Current data and future perspectives on DNA methylation in ovarian cancer (Review)

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Abstract. Ovarian cancer (OC) represents the most prevalent malignancy of the female reproductive system. Its distinguishing features include a high aggressiveness, substantial morbidity and mortality, and a lack of apparent symptoms, which collectively pose significant challenges for early detection. Given that aberrant DNA methylation events leading to altered gene expression are characteristic of numerous tumor types, there has been extensive research into epigenetic mechanisms, particularly DNA methylation, in human cancers. In the context of OC, DNA methylation is often associated with the regulation of critical genes, such as BRCA1/2 and Ras-association domain family 1A. Methylation modifications within the promoter regions of these genes not only contribute to the pathogenesis of OC, but also induce medication resistance and influence the prognosis of patients with OC. As such, a more in-depth understanding of DNA methylation underpinning carcinogenesis could potentially facilitate the development of more effective therapeutic approaches for this intricate disease. The present review focuses on classical tumor suppressor genes, oncogenes, signaling pathways and associated microRNAs in an aim to elucidate the influence of DNA methylation on the development and progression of OC. The advantages and limitations of employing DNA methylation in the diagnosis, treatment and prevention of OC are also discussed. On the whole, the present literature review indicates that the DNA methylation of specific genes could potentially serve as a prognostic biomarker for OC and a therapeutic target for personalized treatment strategies. Further investigations in this field may yield more efficacious diagnostic and therapeutic alternatives for patients with OC.

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Contents

1. Introduction

Ovarian cancer (OC) is one of the three most prevalent and lethal malignancies affecting the female reproductive organs, alongside endometrial and cervical cancers (1). Its incidence has been increasing worldwide, and it now has the second highest yearly incidence rate among cancers of the female reproductive system (2,3). Epithelial OC (EOC), which constitutes 85 to 90% of all ovarian tumors, is the most common subtype (4). The histological subtypes of EOC vary based on the tissue origin, as detailed in Table I. Malignant ovarian germ cell tumors and sex cord-stromal tumors, however, are relatively rare (4). OC is characterized by a combination of direct spread, intraabdominal seeding and lymphatic metastasis, with peritoneal metastases in the advanced stages of the disease being associated with high mortality rates and a poor patient prognosis (5). The primary treatments for patients with advanced-stage OC currently include surgical tumor removal and platinum-based combination chemotherapy (6). Despite advancements in treatment, the prognosis of patients with advanced-stage disease remains poor, and OC continues to have the highest mortality rate among all malignant gynecological malignancies (7). OC often remains asymptomatic in

the early stages due to the covert growth of the ovary, which is the reason why numerous younger women do not experience symptoms from their ovarian tumors. The absence of reliable biomarkers for the early detection of OC often results in the disease progressing to a more difficult-to-treat late stage.

At present, an increasing number of studies have indicated that epigenetic modifications play a pivotal role in tumor growth etiology, and variations in the epigenetic status are emerging as promising non-invasive biomarkers for the early diagnosis and monitoring of OC (8-10). DNA methylation, the most extensively studied and most well-characterized epigenetic modification, regulates gene expression by adding methyl groups to the promoter region of DNA (11,12). DNA methylation is a complex epigenetic modification mediated by a complex network of enzymes, cofactors, and regulatory proteins in a process that involves a variety of channels and receptors that facilitate the interaction between DNA methyltransferases and their targets. These include chromatin remodeling complexes, histone modifiers and transcription factors. In turn, matrix proteins provide the structural framework for enzymes and cofactors involved in methylation and thus play a key role in the process of DNA methylation (10,13). Unlike normal cells, tumor cells often display abnormal DNA methylation levels in specific regions of tumor-suppressor gene and/or oncogene promoters (14,15). This disruption of key biological processes, including cell proliferation, cell cycle regulation and apoptosis, due to the abnormal DNA methylation patterns of certain genes, has been found to be associated with the development of OC (16,17). Recent studies have also suggested that DNA methylation plays a role in OC cell metastasis (11,18). The current understanding posits that DNA methylation markers are crucial in the prevention, diagnosis and treatment of OC, and DNA methylation-related drugs have also exhibited efficacy in reducing or eliminating resistance to chemotherapy and molecular targeting in patients with OC (4,8,19). High-grade serous OC (HGSOC), the most prevalent subtype of OC, has the highest recurrence rate and the worst prognosis. It is widely acknowledged that the primary challenge in treating HGSOC is the acquired resistance to platinum-based drug therapy (20,21). The study by Feng *et al* (22) proposed that NCALD and LAMA3 could serve as novel markers for determining the sensitivity to chemotherapy in patients with HGSOC, and that hypermethylation and the low expression of NCALD and LAMA3 are linked to a poor progression-free survival. It is thus suggested that the methylation of gene promoter regions plays a crucial role in platinum resistance in patients with OC. The present review focuses on the roles of DNA methylation variations in tumor suppressor genes, oncogenes, signaling pathway genes and microRNAs (miRNAs/miRs) involved in the development of OC. Given the challenges posed by drug resistance and relapse mechanisms, which significantly affect the management and prognosis of this disease, the latest findings on the role of DNA methylation in the screening, diagnosis and the treatment of OC are also summarized. The present review comprehensively discusses the current evidence for the role of DNA methylation in both oncogenic and tumor suppressor pathways implicated in OC, in order to identify promising biomarkers or therapeutic targets.

2. Tumor suppressor genes

BRCA1/2. Since the 1990s, studies have often been conducted on BRCA1 and BRCA2 due to their connection to OC. These genes play an essential role in maintaining human health by regulating cellular replication, repairing DNA damage, promoting normal cell development and suppressing tumors (23,24). Since BRCA1 and BRCA2 have complementary roles in protecting against cancer, they are often discussed together. Mutations in BRCA1 and BRCA2, which are crucial genes in the homologous recombination mechanism for the repair of DNA double-strand breaks, have been shown to be associated with an increased risk of developing cancer. Women with a hereditary BRCA1/2 mutation have a higher chance of developing both breast cancer and OC (25). Unlike sporadic breast cancer, BRCA-associated breast cancer is more likely to occur on the side of the body or as a second primary tumor. By the age of 70 years, women with the BRCA1/2 mutation have a 10-59% increased chance of developing OC (26). Therefore, it is critical to conduct additional cancer screening at the time of OC or breast cancer diagnosis and treatment. The increased likelihood of developing primary OC, an earlier onset, larger tumor spread and a more aggressive disease course in BRCA1/2 mutant carriers deserves special attention. Individuals with BRCA1/2 mutations, particularly those with recurrent OC, have an improved prognosis and a longer survival time following surgery compared with individuals with primary and recurrent OC without BRCA1/2 mutations (23,27). This phenomenon was also observed in the study by Yang *et al* (23), in which patients had longer overall survival and progression-free survival times. However, the underlying mechanisms of this difference have yet to be demonstrated, and future studies are required investigate whether it is linked to DNA methylation as a result of BRCA1/2 mutations.

In the study by Jung *et al* (28) comparing the peripheral blood DNA of 55 subjects with no history of cancer and 52 patients with OC, higher rates of BRCA1 methylation were observed in individuals with a family history of cancer, and the presence of BRCA1 methylation increased the risk of developing familial and sporadic EOC. Therefore, BRCA1 methylation testing is an invaluable diagnostic and prognostic tool for individuals who are aware of their BRCA mutation status and have a strong family history. DNA methylation in cervical cells has also been linked to an increased risk of developing OC (29-31). As previously demonstrated, patients with OC who carry the BRCA1/2 mutation have a higher rate of fibrosis and differential methylation in the proximal tubal segment compared with the controls (31). This finding has critical implications for the early detection and treatment of OC.

Clinical trials using poly-ADP ribose polymerase (PARP) inhibitors for the treatment of individuals with BRCA gene mutations are currently underway, since an *in vitro* study demonstrated that cells with mutations in the BRCA1 or BRCA2 genes are particularly sensitive to PARP (32). Additionally, a recent study revealed that females with BRCA methylation were more likely to benefit from treatment with PARP inhibitors, regardless of whether they carried BRCA mutations or not (33). However, further

Table I. Major histotypes of epithelial ovarian cancer.

Categories	Abbreviations	Incidence	Origination	(Refs.)
Serous ovarian cancer (high-grade and low-grade)	HGSOC and LGSOC	60-70%	Fallopian tube epithelium	(3)
Endometrioid ovarian cancer	ENDOC	15%	Endometriosis	(3)
Clear cell ovarian cancer	CCOC	5%	Endometriosis	(3)
Mucinous ovarian cancer	MOC	10%	Transitional cell nests at the tubal-mesothelial junction	(3)

clinical trials are required to confirm the feasibility of the treatment instead. Cisplatin resistance has been linked to the disruption of the BRCA/Fanconi anemia (FA) pathway, which occurs when the FA gene (FA complementation group F) is methylated and silenced (34). Patients with HGSOC commonly exhibit a defective BRCA/FA pathway, rendering the tumor susceptible to DNA cross-linking agents and PARP inhibitors (35). The BRCA1 and BRCA2 genes, are where the majority of BRCA/FA pathway-inactivating mutations are found, particularly in HGSOC (36). Although the DNA methylation of FA complementation group N is rarely observed in HGSOC, it has been shown to be associated with inactivation in some cases of sporadic OC (37). However, targeting the BRCA/FA pathway is expected to overcome OC resistance to cisplatin.

Different subtypes of OC have been found to be associated with distinct patterns of BRCA methylation. HGSOC has been found to have a higher prevalence of BRCA1 hypermethylation compared with other types of EOC (38). The pathophysiology of OC has been linked to the hypermethylation of the BRCA promoter (39). Additionally, Soslow *et al* (40) revealed that BRCA methylation was associated with the presence of solid, pseudo endometrioid and transitional cell carcinoma-like morphology. The combined effect of BRCA1 and BRCA2 hypermethylation in the development of OC supports the use of immune checkpoint inhibitors in clinical trials (41). Furthermore, the methylation of DNA in the upstream region of BRCA1 transcriptional start sites has been observed to positively influence the prognosis of patients with HGSOC (42). Bilateral ovarian cancers are associated with an increased BRCA1 methylation compared with unilateral cancers, and the methylation status can serve as a predictor of the survival of patients with sporadic EOC. The co-expression of DNA methyltransferase (DNMT)1 and 3a, DNMT1 and 3b, or DNMT3a and 3b contributes to the hypermethylation of the BRCA1 promoter, as previously described by Bai *et al* (43). Additionally, Pradjatmo (44) revealed that BRCA2 methylation was present in the majority of patients with OC, and that BRCA2 protein expression levels were associated with overall survival, regardless of the methylation status of BRCA2. This finding may guide the development of therapeutic approaches aimed at preventing or reversing BRCA gene methylation. These results highlight the significance of BRCA methylation in the etiology, progression and prognosis of OC, and lay the foundation for future therapeutic advancements.

The aforementioned evidence suggests that individuals with BRCA1/2 gene mutations, who also exhibit elevated levels of BRCA gene methylation, leading to decreased BRCA1/2 expression, are at a higher risk of developing OC. The methylation testing of the BRCA1/2 gene could potentially play a crucial role in preventing OC. Additionally, individuals with BRCA mutations who have been diagnosed with OC may benefit from treatment that specifically targets BRCA. However, the current findings on the clinical implications of BRCA methylation in OC are complex and conflicting. Therefore, further substantiated data are warranted in order to study and validate the impact of DNA methylation of the BRCA genes in OC.

p53. Both the wild-type and mutant forms of the p53 gene play prominent roles as tumor suppressors in humans. Wild-type p53 is essential for controlling cell division and growth, inducing the apoptosis of malignant cells, and blocking carcinogenesis. By contrast, the mutation of P53 transforms the p53 gene, which is normally a tumor suppressor, into an oncogene that actively promotes cancer development at the cellular level. Point mutations, inactivation and deletions of the p53 gene convert the wild-type to the mutant type, promoting carcinogenesis and cancer progression (45). Studies have revealed a strong association between the methylation of the promoter region of the p53 gene and the onset of several types of cancer, including breast cancer (46), lung cancer (47), prostate cancer (48) and OC (49). A previous study comparing the p53 methylation status in normal and malignant ovarian tissues using methylation-specific PCR determined that the p53 promoter area methylation was unique to OC tissue specimens (50). These results suggest the potential use of p53 promoter area methylation as a screening tool for OC, and indicate that epigenetic modifications play a critical role in OC carcinogenesis.

As illustrated in Fig. 1, yippee like (YPEL)3 is a member of the YPEL1-5 gene family, which encodes putative zinc finger motifs. It is a newly discovered tumor suppressor and p53-regulated gene associated with cellular senescence, causing persistent growth arrest in human tumor and normal cells (51). In ovarian carcinoma cell lines, YPEL3 expression is downregulated due to the hypermethylation of the CpG island upstream of the YPEL3 promoter, resulting in a marked decrease in the suppression of cancer cell proliferation and in the promotion of OC. This role was also confirmed by Kelley *et al* (51) who screened 30 ovarian

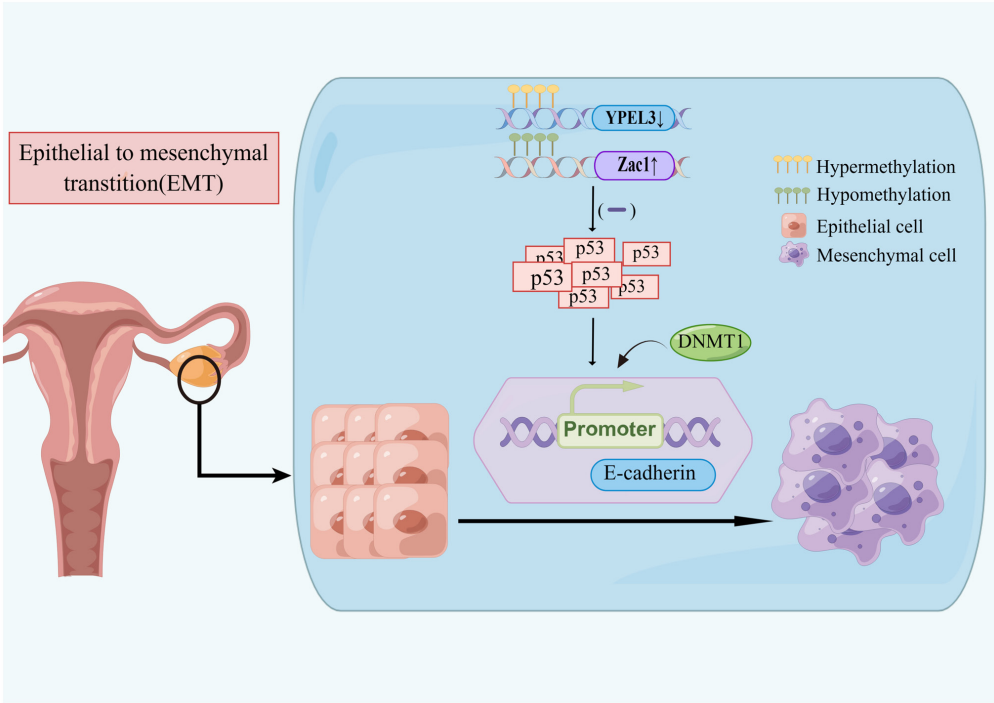

Figure 1. EMT-like changes in ovarian epithelial cells. YPEL3 and Zac1 are regulatory genes for p53, and their altered promoter methylation affects their own expression, which in turn represses p53 expression. The p53-induced downregulation of E-calmodulin expression and DNMT1-mediated promoter methylation are collectively involved in the EMT-like changes in the ovarian epithelium that lead to cancer development. EMT, epithelial-mesenchymal transition; YPEL3, yippee like 3; Zac1, PLAG1 like zinc finger 1; DNMT1, DNA methyltransferase 1.

tumor samples and six normal ovary samples. Additionally, YPEL3 induces cellular senescence downstream of p53, suggesting that upregulating the expression of the YPEL3 gene may be a viable strategy for the treatment of OC (51). Another p53-regulated gene, zinc-finger protein 1 (Zac1), is an imprinted gene expressed in various embryonic and adult somatic organs. By interacting with p53, the tumor suppressor Zac1 can control the transcriptional activity of p53 and induce cell cycle arrest and apoptosis (52). The increased expression of mesenchymal biomarkers and migration support the association between a high expression of Zac1 in cervical cancers and clinical metastasis (53). This is attributed to the hypomethylation of the Zac1 promoter CpG island, leading to the upregulation of its expression in various cervical cancer cell lines (53). Further research is required in order to determine the role of Zac1 in OC, and its involvement in OC development through changes in DNA methylation. In order for non-invasive serous borderline ovarian tumors (SBOTs) to progress to low-grade invasive carcinomas, p53 promotes SBOT invasion by activating the PI3K/Akt pathway and transcription, which in turn suppresses E-calmodulin, as previously demonstrated by Cheng *et al* (54). The downregulation of E-calmodulin by p53 was found to be associated with promoter methylation by DNMT1 (54).

The specificity of the p53 gene results in a complex dichotomy in its role in the pathogenesis of OC. The only studies thus far have shown that p53 is hypermethylated and downregulated in the development of OC. However, there is a lack of studies examining the role of p53 in the epigenetic development of OC, particularly in the context of DNA methylation, despite the association of p53 mutations with an increased risk of developing OC. This presents a key opportunity for further research. Given its potential utility in clinical screening and prognostication of OC, the extensive investigation of the p53 gene methylation is warranted.

Ras-association domain family 1A (RASSF1A). RASSF1A is a potential Ras effector that regulates cellular proliferation and apoptosis in response to extrinsic signals. Its upregulation leads to the decreased proliferation of human cancer cells, indicating its crucial role as a tumor suppressor gene (55). Numerous studies have demonstrated the epigenetic inactivation of the RASSF1A isoform in various types of cancer, such as lung cancer (56), breast cancer (57) and OC (58). Therefore, the methylation of RASSF1A could serve as a valuable prognostic marker for patients with cancer, and may play a critical role in the early detection of cancer (59).

According to recent research, RASSF1A methylation is increased in OC compared with normal ovarian tissues (60). Furthermore, the methylation frequency of RASSF1A has been found to be higher in patients with HGSOC (61). Additionally, the methylation frequency of RASSF1A was higher in OC than in low malignant potential tumors, which exhibited higher methylation levels of RASSF1A than benign ovarian epithelial adenomas (62). Therefore, RASSF1A promoter hypermethylation and RASSF1A protein levels may serve as reliable and sensitive tools for the diagnosis and monitoring of patients with ovarian malignancies. Furthermore, cationic conjugated polymer-based fluorescence resonance energy transfer techniques for the detection of the RASSF1A methylation status in EOC may be useful

for diagnosis and screening. Combining the techniques with the detection of cancer antigen 125 levels may improve the sensitivity of the diagnosis of EOC (63).

RASSF1A promoter methylation has been reported to be markedly associated with EOC in a previous study on OC in Vietnamese women (64). However, a meta-analysis found that the levels of this modification were not substantially linked to the clinicopathological characteristics or the survival outcomes of patients with OC (65). This discrepancy may be due to differences in sample size or individual variations in experimental results. Nevertheless, a recent study examined EOC cells and mesenchymal-like OC stromal progenitor cells to determine their methylation status at RASSF1A promoters (66). The frequency of RASSF1A promoter methylation was found to be considerably higher in tumor-derived OC stromal progenitor cells (OCSPCs) than in epithelial-like OCSPCs, and it was shown to be associated with the clinicopathological characteristics and survival outcomes of patients. That study demonstrated the potential therapeutic value of RASSF1A promoter methylation in OCSPCs generated from EOC tissues (66). Since OCSPCs with a reduced expression of tumor suppressor genes in the ovarian tumor microenvironment can promote tumorigenesis and can be reversed by the DNA demethylation of genes, reversing the DNA demethylation of tumor suppressor genes in OCSPCs may represent a potential therapeutic strategy for OC (67). Reyes *et al* (68) conducted a study on advanced-stage HGSOC and a retrospective, nested, case-control study of patients with recurrent HGSOC. They found that patients with OC in different states had different frequencies of DNA methylation of RASSF1A, and methylation was associated with several differentially expressed genes that could be potential biomarkers and/or therapeutic targets for HGSOC (68).

In patients with advanced-stage EOC receiving neoadjuvant therapy, the methylation status of the RASSF1 promoter has been demonstrated to exhibit a marked association with the response to chemotherapy. Specifically, by studying aberrant DNA methylation in 68 normal ovarian tissues, and 29 benign, 100 malignant and 10 junctional ovarian tumor tissues, Feng *et al* (69) revealed that patients with EOC with RASSF1A promoter methylation had markedly poorer response rates to cisplatin-based neoadjuvant therapy compared with patients without a methylation status. RASSF1A promoter methylation is a key predictive factor for the prognosis of patients with HGSOC (70); the study by Giannopoulou *et al* (71) found that RASSF1A promoter methylation was significantly associated with the OC grade, and that prognosis tended to be worse for patients with OC in whom RASSF1A promoter methylation was detected in the tumor and in adjacent tissues. Furthermore, the identification of aberrant RASSF1A promoter methylation in cell-free circulating tumor DNA from low-volume plasma samples of patients with EOC has shown potential as a prognostic marker for the disease (72). These findings have significant implications for EOC research, including the development of improved diagnostic methods and targeted therapy approaches. In summary, RASSF1A is hypermethylated and downregulated in the development of OC, and the methylation status of RASSF1A has the potential to serve as a biomarker for early identification and diagnosis of OC, as well as for predicting the treatment response and overall clinical outcomes.

Other tumor suppressor genes. In addition to the tumor suppressor genes described above that have undergone substantial research, a large number of other tumor suppressor genes have been linked to the development of OC. Among the principal molecular determinants that exert a profound influence on OC are chromodomain helicase DNA binding 5 (CHD5), fructose-1,6-biphosphatase (FBP1), aldehyde dehydrogenase 1-A2 (ALDH1A1), pluripotency-associated transcription factor forkhead box (FOX)D3, insulin-like growth factor binding protein-3 (IGFBP-3), zinc finger protein 671 (ZNF671), secreted protein acidic and rich in cysteine (SPARC) and O^6-methylguanine-DNA methyltransferase (MGMT). All the aforementioned tumor suppressor genes can be accessed from Table II.

CHD5, also known as DNA binding 5, is a member of the subclass of chromatin remodeling Swi/Snf proteins and is currently considered to be a tumor suppressor (73). The frequency of abnormal DNA methylation of the CHD5 gene promoter is inversely related to the prognosis of patients with cancer and has been observed in several malignancies (74-78). Despite the limited number of studies, CHD5 promoter methylation has been shown to be associated with OC, suggesting its potential clinical applications in OC metastasis, treatment and prognosis. The downregulation of FBP1, a tumor suppressor and the rate-limiting enzyme in gluconeogenesis (79), has been observed in several malignancies. The DNA methylation of the FBP1 promoter in patients with OC leads to a decreased expression of FBP1, which is associated with advanced-stage disease, high malignancy, low survival, high recurrence rates, and a poor prognosis (80). Compared to the normal ovarian surface epithelium, OC cells have a significantly lower expression of ALDH1A2, another rate-limiting enzyme involved in cellular retinoid production (81). High ALDH1A2 promoter methylation levels in OC cells promote cell proliferation, enhance invasive activity and are associated with a poor prognosis (82). Together, CHD5, FBP1, and ALDH1A2 have exhibited promise as biomarkers, therapeutic targets and prognostic indicators in the study of OC and its clinical management.

FOXD3 is essential for development, cellular homeostasis and the control of lineage specification (83). The reduced expression of FOXD3 due to the hypermethylation of its promoter has been linked to the development of malignant tumors (84,85). The study by Luo *et al* (86) revealed that FOXD3 promoter methylation was increased and its expression was decreased in OC tissues. The inhibition of tumor cell growth, as well as the effects on tumor cell proliferation and migration, suggest that FOXD3 promoter methylation may serve as a prognostic marker for OC (86). IGFBP-3 is a member of the IGFBP family, which largely governs the mitogenic and anti-apoptotic effects of insulin-like growth factor, a protein whose transcription is regulated by p53 and which possesses anti-proliferative, pro-apoptotic and invasion-inhibiting activities (87). Wiley *et al* (88) discovered that changes in IGFBP-3 promoter methylation

Table II. Tumor-associated suppressor genes with abnormal DNA methylation in ovarian cancer.

Genes	Alternate gene name	OC subtypes	DNA methylation	Gene expression	(Refs.)
BRCA1/2	Breast cancer susceptibility gene	HGSOC	High	Low	(32,38)
p53	Tumor protein p53	HGSOC	High	Low	(44)
RASSF1A	Ras association domain family member 1	HGSOC	High	Low	(54,55)
CHD5	Chromodomain helicase DNA binding protein 5	/	High	Low	(73)
FBP1	Fructose-1,6-bisphosphatase	/	High	Low	(74,75)
ALDH1A2	Aldehyde dehydrogenase 1 family, member A2	HGSOC	High	Low	(81,82)
FOXD3	Forkhead box D3	/	High	Low	(83-86)
IGFBP-3	Insulin-like growth factor binding protein 3	EOC	High	Low	(87,89)
ZNF671	Zinc finger protein 671	HGSOC	High	Low	(92)
SPARC	Secreted protein acidic and rich in cysteine	/	High	Low	(93,94,97)

significantly affected the survival of patients with EOC. This link was found to be particularly strong in individuals with early-stage OC. Furthermore, in patients with EOC who lacked p53 overexpression, elevated DNA methylation levels of the IGFBP-3 promoter were found to be substantially associated with OC progression (89). ZNF671, a member of the KRAB-ZFP family that contains C2H2-type zinc fingers and a Krüppel associated box domain, regulates key functions in cell differentiation, proliferation, apoptosis and tumor suppression (90,91). The low expression of ZNF671 is strongly associated with OC cell motility and invasion, and it is one of the most heavily methylated genes in patients with early recurrence. The ZNF671 DNA methylation status following platinum-based adjuvant chemotherapy may be a potent indicator of serous OC recurrence (92). In summary, promoter hypermethylation affects the low expression of FOXD3, IGFBP-3 and ZNF671, which contributes to the onset and progression of OC. Patients with early-stage OC may benefit from using these genes as prognostic indicators. If other members of this family are found to be involved in the formation of OC, which is yet to be determined, scholars can explore additional gene targets that can aid in the clinical diagnosis and therapy of OC.

To regulate cell adhesion, differentiation, proliferation, migration, tissue remodeling, morphogenesis and angiogenesis, SPARC (also known as osteonectin or BM-40) is expressed in various types of cells (93). Previous studies have linked the hypermethylation of the SPARC gene promoter to a worse prognosis and earlier diagnosis in several types of cancer, including OC (93-95). The SPARC promoter is methylated in primary OC and that SPARC protein levels decrease as the disease advances from low to high grade (95-97). Based on these results, it appears that SPARC promoter methylation plays a critical role in OC carcinogenesis and survival, and SPARC may serve as a novel biomarker for OC.

Comparative research has been conducted on the role of MGMT, a DNA repair gene that is hypermethylated in the majority of malignancies (98). The frequency of MGMT gene promoter methylation varies across OC samples, with EOC having the highest frequency and benign ovarian tissue having the lowest (99). The role of MGMT promoter methylation in the onset of OC is undeniable, despite the lack of clarity regarding the link between MGMT gene expression and DNA methylation.

DNA hypermethylation and the low expression of the aforementioned genes is strongly associated with OC etiology and has significant implications for the treatment and prognosis of patients with OC. Targeting these genes could greatly benefit the early screening, diagnosis and therapy of patients with OC. It is crucial to gather convincing data to identify their precise role in OC and uncover their latent potential in managing this malignancy, considering the large number of genes present in the human genome and the need to discover more tumor suppressor genes.

3. Oncogenes

The atypical expression of oncogenes, which can be caused by epigenetic alterations, has been found to be associated with tumor development and a poor prognosis. The significance of oncogenes in the pathogenesis of OC is demonstrated by the identification of ~568 oncogenes, of which ~34 are associated with the risk of developing OC. Common epigenetic abnormalities include alterations in DNA methylation, RNA interference, histone modifications and gene mutations (100). The present review focuses on five oncogenes: Homeobox A9 (HOXA9), chromobox protein homolog 8 (CBX8), solute carrier family 6, member 12 (SLC6A12), anterior gradient 2 (AGR2), and gamma-aminobutyric acid (GABA) A receptor subunit (GABRP), due to their known association with OC and abnormal DNA methylation (Table III).

The DNA-binding transcription factor, HOXA9, controls gene expression and plays a role in morphogenesis and differentiation (101). The methylation of the HOXA9 promoter has been extensively studied in relation to the development of OC (102). Widschwendter *et al* (102) observed that HOXA9 promoter methylation in normal endometrium increased the incidence of OC by 12.3-fold across all stages and 14.8-fold in early-stage OC, independent of age, menstrual cycle and cancer histology. Wu *et al* (103) revealed that HOXA9 promoter hypermethylation was more common in older

Table III. Tumor-associated oncogenes with abnormal DNA methylation in ovarian cancer.

Oncogenes	Alternate gene name	OC subtype	DNA methylation	Gene expression	(Refs.)
HOXA9	Homeobox A9	HGSOC	Low	High	(103,105-107)
CBX8	Chromobox protein homolog 8	/	Low	High	(109)
SLC6A12	Solute carrier family 6, member 12	/	Low	High	(111)
AGR2	Anterior gradient 2	EOC	Low	High	(112,114,115)
GABRP	γ-aminobutyric acid (GABA) A receptor π subunit	HGSOC	Low	High	(148)

women and was associated with a higher frequency of methylation in the early stages of OC by examining 52 primary OCs and their *in vitro* models. Montavon *et al* (104) found that the HOXA9 promoter was differentially methylated in primary HGSOC and rarely methylated in benign ovarian surface epithelium (OSE), suggesting that the combination of HOXA9 promoter methylation status could distinguish HGSOC from benign OSE with a sensitivity of 100% when pre-operative CA125 levels were also included. Studies have also demonstrated that HOXA9 promoter methylation is highly tumor specific and has great promise as a diagnostic serum biomarker for the early screening of OC (95,105). The diagnostic utility of promoter methylated HOXA9 in circulating tumor specific DNA in patients with OC was examined by Faaborg *et al* (106), who deviated from the standard practice of studying DNA methylation by analyzing both the sense and antisense strands of the HOXA9. They discovered that compared to single-stranded assays, OC diagnostics could benefit from simultaneous testing against both DNA strands, leading to a 59.5% increase in sensitivity (106). In addition, HOXA9 promoter methylation was previously found to be involved in the progression from one grade of OC to another. For example, in patients with endometriosis-associated OC, lower levels of HOXA9 promoter methylation were significantly associated with a higher tumor grade. This suggests that the HOXA9 promoter methylation pattern is an indicative factor for progression toward high-grade plasmacytoma (96). Furthermore, the promoter hypermethylation of HOXA9 can be used as a diagnostic marker and can also be used to forecast prognosis of patients. Patients with platinum-resistant BRCA-mutated OC treated with PARP inhibitors have been shown to have a poor prognosis if their HOXA9 promoter is highly methylated (107). This suggests that HOXA9 promoter hypermethylation and the low expression of HOXA9 in OC can be a valuable predictive biomarker and can inform clinical decision-making in platinum-resistant BRCA. Overall, these results highlight the potential of HOXA9 methylation as a diagnostic marker for OC, with applications in risk prediction and prognosis forecasting.

CBX8 is a fundamental CBX protein and maintains pluripotency and self-renewal during developmental program controls, cell destiny determinations and the regulation of embryonic stem cells. Cell cycle progression, senescence and differentiation are all influenced by CBX8, and the hypomethylation of its promotor leads to an increase in its expression (108). CBX8 has been shown to play a role in the development of hepatocellular carcinoma, renal cancer and colorectal cancer, among others (109,110). The DNA hypomethylation of CBX8 leads to an enhanced expression, which acts as a potential diagnostic and prognostic biomarker for patients with OC and is associated with a poor prognosis (109). Furthermore, SLC6A12, a betaine/GABA transporter (111), is overexpressed in OC metastases, negatively affecting patient survival due to its promoter hypomethylation. Since SLC6A12 promoter methylation facilitates cancer cell invasion during the development of OC, it is widely recognized as a prognostic marker for the chances of survival of patients (111).

AGR2, a protein disulfide isomerase localized to the endoplasmic reticulum or secreted into the extracellular space, has been linked to cancer progression in patients with similar tumors (112-114). According to the study by Sung *et al* (115), which used a mouse model of human OC metastasis, the CpG site in the promoter region of AGR2 is hypermethylated in metastatic tumor tissue, which typically results in AGR2 overexpression. AGR2 overexpression was found to increase SK-OV-3 cell migration and invasion (115). This suggests that AGR2 promoter hypomethylation may contribute to OC cell metastasis and invasion.

There are other studies on gene mutations in OC subtypes, with previous studies finding that HGSOC has prevalent TP53 mutations, mucinous OC has frequent KRAS mutations, and mutations in AT-rich interaction domain 1A and phosphatidylinositol-4,5-bisphosphate 3-kinase (PIK3)CA are more common in clear-cell OC and endometrioid OC (35). However, studies exploring epigenetic modifications, particularly DNA methylation, in OC subtypes are limited. A genome-wide DNA methylation analysis revealed that HGSOC exhibited higher levels of overall DNA hypermethylation compared to low-grade EOC (116). DNA methylation profiles could potentially be utilized to predict and classify the characteristics of aggressive and high- or low-grade EOC. HGSOC, which typically displays a higher overall DNA methylation, is often associated with varying degrees of platinum resistance, leading to differing recurrence intervals following initial paclitaxel/platinum-based therapy (116). The data reviewed above suggest that oncogene promoter hypomethylation is a common mechanism leading to oncogene overexpression in patients with OC. Previous studies support the hypothesis that oncogenes contribute to OC cell metastasis and have significant clinical implications for the diagnosis and prognosis of patients with OC (102-106). However, the possible role of

Table IV. Wnt/β-catenin signaling pathways with abnormal DNA methylation in ovarian cancer.

Genes	Alternate gene name	DNA methylation	Gene expression	(Refs.)
SFRP5	Secreted frizzled-related protein 5	High	Low	(124,126,127)
IQGAP2	IQ motif containing GTPase activating proteins 2	High	Low	(128,129)
TMEM88	Transmembrane protein 88	High	Low	(130,131)

aberrant oncogene promoter methylation in the therapy and acquired drug resistance of patients with OC is poorly studied, making it a promising area for further research.

4. Pathways

Wnt/β-catenin signaling pathway. Wnts are a class of glycoproteins that primarily exert their effects through autocrine or paracrine secretion. Upon secretion, they interact with surface receptors, triggering a cascade of downstream protein phosphorylation and dephosphorylation events that ultimately result in the accumulation of β-catenin. Adhesive bands are formed when β-catenin interacts with E-cadherin at cell junctions. Free β-catenin can enter the nucleus to influence gene expression. The aberrant expression or activation of β-catenin can lead to the development of cancer (117). Several types of cancer, including lung cancer (118), breast cancer (119) and OC (120), have been shown to share a common feature: The oncogenic activation of the Wnt/β-catenin signaling pathway. Various component alterations (Table IV) in the Wnt/β-catenin signaling pathway and their significance in OC are discussed below.

Secreted frizzled-related proteins (SFRPs) play a crucial role in cancer progression and prognosis by functioning as critical inhibitors of the Wnt/β-catenin signaling pathway. The SFRP (SFRP1, 2, 3, 4 and 5) genes are heavily methylated, leading to transcriptional silencing (121). This downregulation of SFRP expression is a common occurrence in cancer. In OC, the SFRP1 gene is inactivated due to promoter methylation and participates in the Wnt/β-catenin signaling pathway (122). Promoter hypermethylation also contributes to the inactivation of SFRP5, disrupting Wnt/β-catenin signaling and promoting ovarian carcinogenesis (123). Patients with SFRP5 promoter methylation have a poorer prognosis (124,125). Moreover, SFRP5 hypermethylation is associated with an increased risk of EOC recurrence and mortality, suggesting its potential as a prognostic biomarker (126). SFRP5 expression also suppresses epithelial-mesenchymal transition (EMT) and increases the sensitivity of OC cells to chemotherapy (127). Conversely, SFRP5 hypermethylation in OC leads to the oncogenic activation of the Wnt/β-catenin pathway, resulting in an increased OC progression and chemoresistance through TWIST-mediated EMT and AKT2 signaling (127). Curcumin, a targeted anticancer agent, inhibits the Wnt/β-catenin signaling, thereby mitigating the effects of SFRP5 hypermethylation. When used in combination with 5-aza-2'-deoxycytidine, it attenuates the development of OC (123). Apart from SFRP5, there is limited research available onSFRP1/2/3/4, and further investigations are required to understand the potential of targeting SFRP and inhibiting the Wnt/β-catenin signaling pathway for OC.

IQ motif containing GTPase activating protein (IQGAP)2, a member of the IQGAP family, functions as a tumor suppressor in the majority of cancers by mainly inhibiting β-catenin nuclear translocation and transcriptional activity. This inhibition leads to the suppression of Wnt/β-catenin signaling, which in turn inhibits the EMT, migration and invasion of OC cells. In OC, the DNA methylation of IQGAP2 is significant and negatively correlates with mRNA expression. Survival analyses have revealed that a reduced expression of IQGAP2 is strongly associated with a poorer progression-free survival of patients with OC (128,129). Another new protein, transmembrane 88 (TMEM88), is an inhibitor of Wnt signaling and is found in the cell membrane (130). Promoter hypermethylation causes a decrease in TMEM88 expression, which enhances OC cell proliferation and the development of resistance to platinum treatments (131). The tumor suppressive functions of IQGAP2 and TMEM88 in OC are mediated through the regulation of Wnt/β-catenin signaling, resulting in reduced cell proliferation and invasion. These findings provide insight into the pathophysiology of OC and suggest potential therapeutic interventions for this condition. Additionally, IQGAP2 and TMEM88 may serve as useful biomarkers for the diagnosis and monitoring of OC.

The role of DNA methylation in OC in the regulation of the Wnt/β-catenin signaling pathway is illustrated in Fig. 2. The aforementioned results demonstrate that hypermethylation and the low expression of key genes in the Wnt/β-catenin signaling cascade can significantly affect OC pathogenesis, particularly in the context of therapy and platinum resistance in patients with OC. Therefore, it is crucial to continue studying the Wnt/β-catenin signaling pathway due to its potential in combating platinum resistance in patients with OC.

Transforming growth factor (TGF)-β signaling pathway. There are three closely comparable structural isoforms of TGF-β, all of which belong to the same family of cytokines. The TGF-β signaling pathway has been shown to play a bidirectional role in cancer progression and is essential for regulating cellular activities, such as cell proliferation, differentiation, apoptosis and cellular dynamic homeostasis (132). In the early stages, it functions primarily as a tumor suppressor, while in advanced stages, it may function as a tumor promoter (133). As demonstrated in the study by Matsumura *et al* (134), DNMT inhibitors (DNMTis) can enhance TGF-β pathway activity and reduce the progression of OC. Furthermore, a follow-up study revealed that TGF-β therapy causes changes in DNA methylation

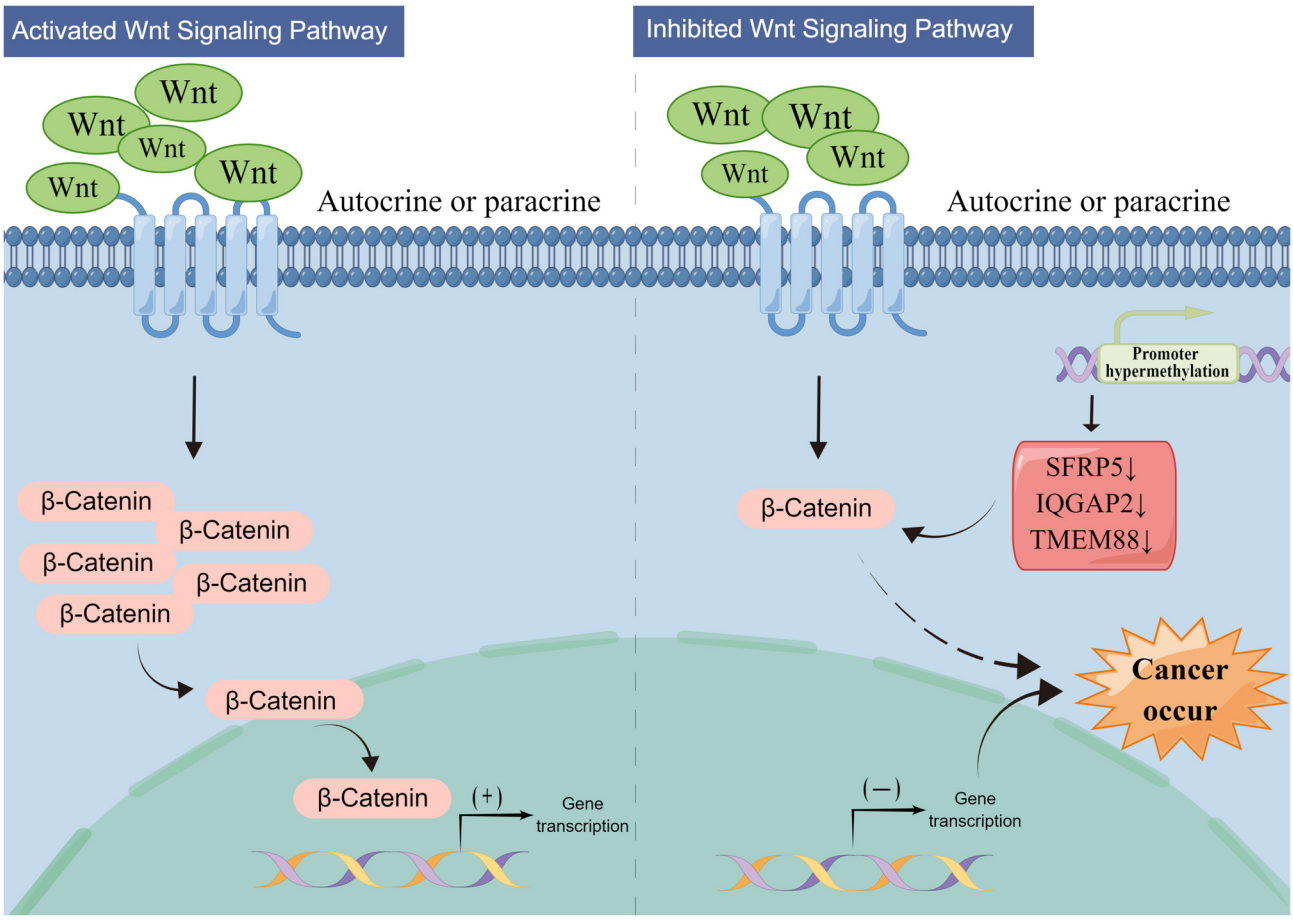

Figure 2. Activation and inhibition of the Wnt/β-catenin signaling pathway. SFRP5, IQGAP2 and TMEM88 are some of key genes in the Wnt/β-catenin signaling pathway. The hypermethylation of their promoters leads to the downregulation of their expression, which in turn leads to the aberrant expression of β-catenin. This leads to the inhibition of the Wnt/β-catenin signaling pathway and the development of cancer. SFFRP5, secreted frizzled related protein 5; IQGAP2, IQ motif containing GTPase activating protein 2; TMEM88, transmembrane protein 88.

that persist throughout the EMT phase of OC cells. Notably, blocking TGF-β from inducing EMT in cancer cells with DNMTi therapy reduced cancer cell metastasis (135). These results suggest that DNMTis may be a promising therapeutic option for OC by regulating the TGF-β signaling pathway and preventing cancer cell metastasis.

F-box protein 32 (FBXO32), a member of the F-box protein family, is highly methylated in advanced OC, resulting in decreased expression. Since FBXO32 is a target gene of SMAD4, its loss in OC causes a malfunction in the TGF-β/SMAD4 signaling pathway, accelerating the development of OC (136). However, OC cells can become desensitized to cisplatin, and their expression of FBXO32 can be restored by treatment with epigenetic drugs, which also markedly reduces the growth of platinum-resistant OC cell lines *in vitro* and *in vivo*. Additionally, the methylation status of FBXO32 can predict the survival of patients with OC (136). ATP binding cassette subfamily A member 1 (ABCA1), a signaling target of TGF-β, is also expressed as DNA hypermethylation in OC, and the higher the methylation level of ABCA1 promotor, the higher the pathological grade and the shorter the survival of patients with OC (137). Sex-determining region Y-box 2 (SOX2) is a single-exon transcription factor with key roles in embryonic development and stem cell maintenance (138). Shonibare *et al* (139) observed an improved lifespan of tumor-bearing mice following the promoter methylation of SOX2. This suggests that the promoter methylation of SOX2 can influence the TGF-β signaling pathway, which in turn affects the survival of patients with OC and the metastasis of OC cells (139). TGF-β-induced protein (TGFBI), also known as βig-H3 and keratoepithelin, is a cellular matrix protein whose promoter hypermethylation is associated with the silencing of TGFBI. This can induce OC cell death and is significantly associated with the development of OC (140). Wang *et al* (141) confirmed this and also found that the hypermethylation of TGFBI was associated with paclitaxel resistance in patients with OC. Therefore, they hypothesized that TGFBI may be a therapeutic target for improving the chemotherapeutic response in patients with OC (141). Overall, the expression of FBXO32, ABCA1, SOX2 and TGFBI, which are genes in the TGF-β signaling system, is suppressed due to hypermethylation, thereby accelerating the development of OC. The methylation profiles of these genes (Table V) can be utilized to predict the prognosis of patients with OC and can also be targeted for therapeutic purposes against the disease.

Other pathways. The abnormal activation of the PI3K/AKT/mammalian target of rapamycin (mTOR) signaling pathway is very common occurrence in the majority of human cancers compared to other major signaling pathways.

Table V. TGF-β signaling pathways with abnormal DNA methylation in ovarian cancer.

Genes	Alternate gene name	DNA methylation	Gene expression	(Refs.)
FBXO32	F-box protein 32	High	Low	(136)
ABCA1	ATP binding cassette subfamily A member 1	High	Low	(137)
SOX2	Sex-determining region Y-box 2	High	Low	(139)
TGFBI	Transforming growth factor-beta-induced protein	High	Low	(140,141)

The inactivation of the phosphatase and tensin homolog (PTEN) gene often occurs at an early stage in ovarian endometrioid and ovarian clear cell carcinomas, and its promoter is often methylated in 40% of ovarian clear cell adenocarcinomas (142), which can negatively regulate the PI3K/AKT/mTOR signaling pathway. Moreover, PTEN also plays a role as an oncogene (143). Li *et al* (144) found that the hypomethylated PIK3R3 promoter was detected in OC cell lines, which may play a role in the chemoresistance of OC, and can even restore sensitivity to platinum-based chemotherapeutic agents. Overall, the current focus of studies on genetic abnormalities in the PI3K/AKT/mTOR signaling pathway is on genetic mutation abnormalities; however, epigenetic regulation, particularly DNA methylation, has been less extensively studied and warrants further investigations. The MAPK pathway is another currently well-studied pathway that is aberrantly activated during tumor progression and is present in >85% of cancers (145). Human growth factor receptor-bound protein-7 (GRB7), is overexpressed in a variety of human cancers (146,147). A recent study by Chen *et al* (147) found that miR-193a-3p directly regulated GRB7 and that miR-193a-3p was downregulated by DNA hypermethylation during the development of OC, leading to the elevated expression of GRB7 in OC tissues. In addition, miR-193a-3p enhances the oncogenicity of OC cells by regulating Erb-B2 receptor tyrosine kinase (ERBB)4, SOS Ras/Rho guanine nucleotide exchange factor 2 and KRAS in the MAPK/ERK signaling pathway (147). Therefore, miR-193a-3p and GRB7 are promising as targets in OC therapy and deserve further exploration. The experimental study of Sung *et al* (148) found that the CpG site of the GABRP promoter was hypomethylated in the metastatic tissues of mice with tumor xenografts, leading to the overexpression of GABRP, and the promotion of cell migration and invasion through the activation of the MAPK/ERK pathway. This suggests that GABRP enhances the invasive phenotype of OC cells and that the DNA methylation status of the GABRP-963 CpG locus may help predict the metastatic potential of patients with OC (148). The use of animal models well reflects the physio-pathological mechanisms in the human body and helps to assist the target therapy of human diseases; however, ultimately, research has to be returned to the human body for validation, in order to provide more realistic and accurate results for clinical treatment.

Although there are more studies on signaling pathways in OC, the focus of the studies is on the mutation or inactivation of key genes, and studies on epigenetic modifications are limited, which has some limitations. Therefore, further experimental studies are required for validation, and they are expected to promote the advancement of OC as a disease in chemotherapy-resistant treatment and prognostic assessment.

5. MicroRNAs and DNA methylation

Role of miRNAs in the diagnosis and treatment of OC. miRNAs are a class of short, non-coding RNAs that function by simultaneously repressing translation and/or causing RNA degradation by targeting numerous mRNAs. Previous research has shown that OC tissues have distinct miRNA expression profiles from those of normal human ovarian tissues (149,150). DNA methylation at the promoter of the host gene controls the expression of numerous miRNAs. The methylation alterations of genes associated with miRNAs in the development of OC are summarized in Table VI. Cancer cells rely on a specific type of energy metabolism known as the Warburg effect, which is partially controlled by miRNAs (151). miR-532-3p and miR-145, which are overexpressed in OC tissues, have been shown to prevent the Warburg effect in OC cells (152,153) and exhibit a negative correlation with DNMT3A expression. More specifically, miR-145 predominantly operates via the miR-133b/pyruvate kinase M2 pathway to induce the Warburg effect (154). The hypomethylation of the DNMT3A/3B CpG island promoter area enhances miR-29b expression, and there is an inverse association between miR-29b and DNMT3A/3B expression levels in OC tissues. The therapeutic targeting of miR-29b may represent a promising new avenue for the management of OC (155).

The secretory epithelial cells of the fallopian tube (FTSECs) play a crucial role in the maturation of HGSOC. The malignant transformation of FTSECs is more common following long-term exposure to iron. Chhabra *et al* (156) found that the expression of miR-432-5p and miR-127-3p was considerably downregulated during this malignant transformation. Chronic exposure to iron can affect miRNA expression by causing epigenetic modifications; however, this effect can be reversed by treatment with DNA methyltransferase inhibitors (156). The tumor suppressor gene, DNMT1/UTF1, is also downregulated by miR-148a-3p, which has been found to reduce cancer cell proliferation (157). Based on these results, miRNAs may be useful as diagnostic indicators and therapeutic targets for treating OC.

miRNAs have also been linked to platinum resistance, which is a well-known and challenging barrier in the treatment of OC with chemotherapy. The low expression of miR-509-3p and a significantly higher frequency of miR-509-3p methylation have been shown to be associated with a shorter overall survival of OC cells derived from patients who

Table VI. MicroRNA-associated gene methylation alterations in ovarian cancer.

MicroRNA	DNA methylation	Gene expression	Major target or pathway	Clinical function	(Refs.)
miR-532-3p	Low	High	HK2	Warburg effect	(152)
miR-145	Low	High	miR-133b/PKM2	Warburg effect	(154)
miR-29b	Low	High	DNMT3A/3B	Treatment	(155)
miR-152	High	Low	DNMT1	Treatment	(191)
miR-148a	High	Low	DNMT1	Treatment	(191)
miR-193a-3p	High	Low	GRB7, DNMT1/UTF1	Proliferation	(147)
miR-509-3p	High	Low	COL11A1, SUMO-3	Invasion and chemical sensitivity	(158)
miR-30a-5p and miR-30c-5p	High	Low	DNMT1	Cisplatin resistance	(159)
miR-143	High	Low	DNMT3A	Cisplatin resistance	(164)
miR-199a-3p	High	Low	DDR1	Prognosis	(168)
miR-34	High	Low	P53	Grading and prognosis	(173)
miR-125b	High	Low	ERBB2 or ERBB3	FIGO stage	(170)

HK2, hexokinase 2; miR, microRNA; PKM2, pyruvate kinase M2; DNMT, DNA methyltransferase; GRB7, growth factor receptor-bound protein-7; UTF1, undifferentiated embryonic cell transcription factor 1; COL11A1, collagen type XI alpha 1 chain; SUMO-3, small ubiquitin like modifier 3; DDR1, discoidin domain receptor 1; ERBB, Erb-B2 receptor tyrosine kinase.

have undergone primary tumor cytoreductive surgery and post-operative platinum-based chemotherapy (158). This association is primarily mediated by collagen type XI alpha 1, which increases the phosphorylation and stability of DNMT1 (158). In cisplatin-resistant OC cells, the overexpression of DNMT1 induces methylation and the subsequent downregulation of miR-30a-5p and miR-30c-5p, resulting in cisplatin resistance (159). The poor prognosis of patients with OC corresponds with the dysregulation of miR-7, miR-132, miR-335 and miR-148a in cisplatin-resistant cell lines, where miR-7 tends to exhibit specific methylation and is associated with a worse prognosis of patients with OC (160). Restoring miR-9 expression by demethylating the miR-9-1/3 gene can desensitize OC cells to paclitaxel (161). The downregulation of miR-9 expression in paclitaxel-resistant EOC cells is related to resistance to paclitaxel. It has also been found that promoter hypermethylation in OC tissues reduces the expression of miR-479 and miR-130b, decreasing the sensitivity of cancer cells to platinum-based therapies (162,163). Recent studies have reported a newly discovered substance, miR-143, which has been proven to play a role in the chemotherapy of tumors. DNMT3A is a direct target of miR-143, and the overexpression of DNMT3A antagonizes the sensitivity of miR-143 to cisplatin in OC cells, possibly as DNMT3A leads to the hypermethylation of the miR-143 precursor gene, resulting in the downregulation of its expression and generating cisplatin resistance (164).

All these points emphasize the importance of miRNAs in the therapeutic intervention of OC, specifically in relation to the reported link between miRNAs and DNA methylation. This notable finding suggests potential new avenues for the treatment of OC, and further research is required to determine its therapeutic implications.

Roles of miRNAs in the staging and progression of OC. In addition to their roles in diagnosis and treatment, miRNAs also play crucial roles in the progression of OC. Previous research has demonstrated that the expression of total miRNAs can be used to reliably distinguish between normal and malignant cells, and that miRNAs are abnormally expressed in human OC compared to normal ovaries (165). Among the miRNAs examined, miR-141, miR-200a, miR-200b and miR-200c were found to be significantly overexpressed, while miR-199a, miR-140 and miR-145 were significantly downregulated in OC tissues (166). Furthermore, the overexpression of miR-21, miR-203 and miR-205 in OC tissues, as opposed to normal tissues, may be attributed to DNA hypomethylation, which has been observed following the treatment of OVCAR3 cells with 5-aza-2'-deoxycytidine demethylation (166). Additionally, the hypermethylation of miRNAs has been found to be associated with a shorter survival rate of patients with OC, and the expression of miRNAs, particularly that of miR-203a-3p, has been shown to be significantly reduced in OC metastatic tumors (167). Knockdown of DNMT increases the expression of miR-199a-3p, and the level of miR-199a-3p promoter methylation is also significantly elevated in OC cells (168). The overexpression of miR-199a-3p leads to a decrease in the expression of discoidin domain receptor 1, which subsequently reduces the migration and invasiveness of OC cells (168). Moreover, the miR-34 family has been shown to possess tumor suppressive properties that mediate apoptosis and promote cellular senescence; however, its expression in OC cells is significantly reduced, primarily due to the methylation of miR-34a CpG islands. This downregulation of miR-34a expression affects the grading and prognosis of patients with OC (169). Zuberi *et al* (170) reported that DNA hypermethylation may be involved in the inactivation of miR-125b,

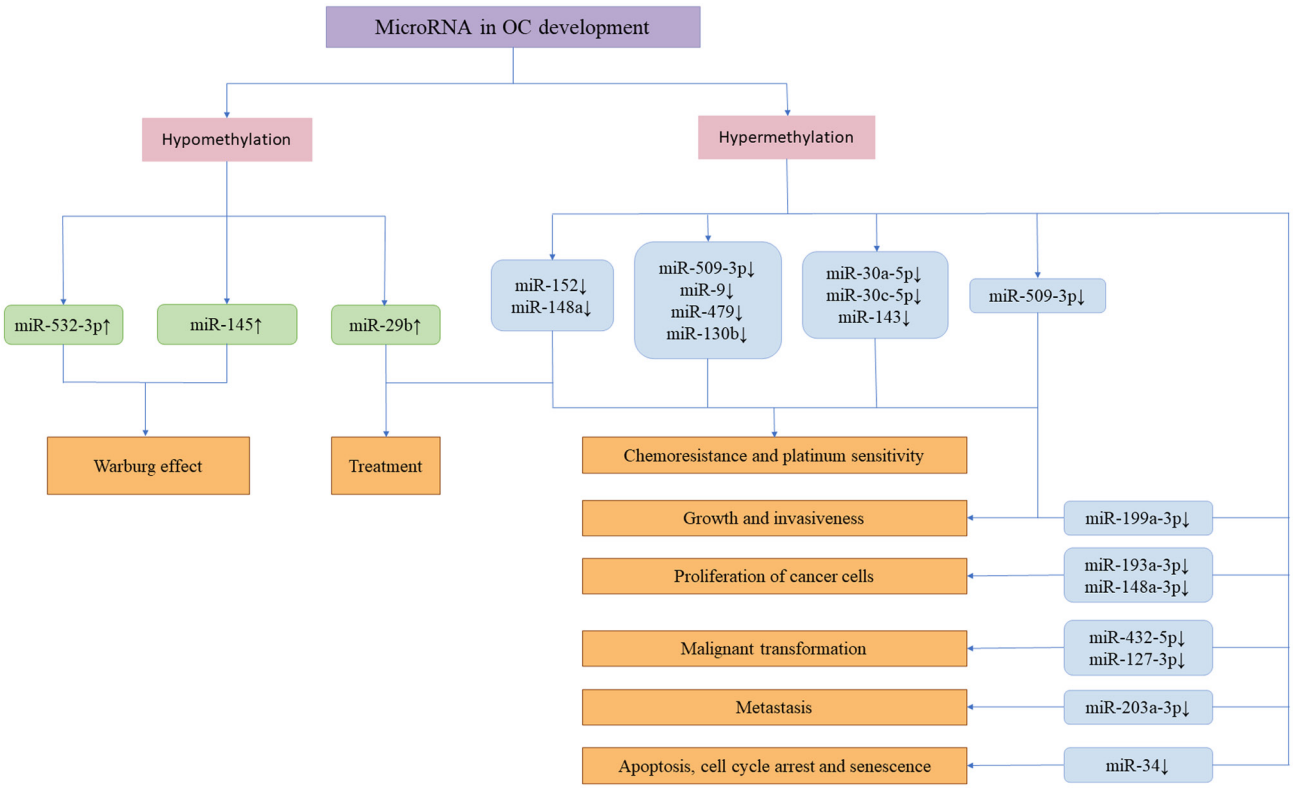

Figure 3. Altered methylation of related genes in miRNAs and changes in their expression, with the role they play in OC. OC, ovarian cancer; miR/miRNA, microRNA.

and miR-125b was shown to be significantly associated with FIGO staging and the metastasis of OC. The overexpression of ERBB2 or ERBB3 is known to be associated with cancer development and poor prognosis. He *et al* (171) demonstrated that reactive oxygen species inhibited the expression of miR-199a and miR-125b by increasing the promoter methylation of the miR-199a and miR-125b genes through DNMT1. This led to changes in the expression levels of ERBB2 and/or ERBB3 in OC cells, thereby attenuating the progression of OC (171).

It has been reported that the overexpression of tet methylcytosine dioxygenase 3 (TET3) can reverse TGF-β1-induced EMT-like changes, mainly by demethylating the promoter of the precursor gene of miR-30d. Thus, there is an association between TET3 and the grade of differentiation of OC, and TET3 plays a role in suppressing the progression of OC (172). miR-34a and miR-34b/c are direct target genes of p53 and have tumor suppressor properties, as they mediate apoptosis, cell cycle arrest and senescence (169). However, the inactivation of miR-34 in OC suggests that the CpG methylation of miR-34a and miR-34-b/c may be of diagnostic value. The mutual exclusivity of miR-34a methylation and p53 mutations suggests that the inactivation of miR-34a may substitute for the loss of p53 function in cancer and induce the proliferation of OC cells (173). Therefore, conducting in-depth studies on miRNAs may be beneficial for the further elucidation of the pathogenesis of OC.

As illustrated in Fig. 3, miRNAs have attracted attention as potential biomarkers for early identification and prognostic evaluation, due to their roles in disrupting DNA methylation and gene targeting in the pathogenesis of OC. Moreover, miRNAs have shown promise as therapeutic targets for the treatment of patients with recurrent OC with acquired medication resistance. Further research is necessary to fully understand the mechanisms involved in this area.

6. Prospects for the clinical application of DNMT inhibitors in OC

The expression levels of DNMTs in various ovarian tissues have been found to be highly associated with the pathology and survival outcomes of patients with OC. The 15-spliced protein product or isoform encoded by DNMT3B is essential for the migration and invasion of OC (174). DNMT3B has been shown to methylate retinol binding protein 1, which has both oncogenic and autophagic effects in OC cells (175). The inhibitory effects of TET3 on the migration and invasion of OC can be diminished by DNMT3B binding to the TET3 promoter, resulting in methylation of the promoter region (176). Therefore, blocking DNMT3B can reduce the growth, migration and invasion of OC cells. However, in HGSOC, DNMT3B1 and DNMT3B3 are overexpressed, and the overexpression of DNMT3B3 leads to the marginal gene demethylation of OVCAR3 human OC cells, which is associated with a poor prognosis (177). DNMTis, which are chemically similar to deoxycytidine, have been shown to block methyl transfer by inhibiting DNMT activity. Previous studies have confirmed the potential therapeutic benefits of targeting DNMT in OC, leading to better clinical outcomes and prognoses (174,178).

Through a process of viral sensing, DNMTis can also induce the production of endogenous retroviruses, which in turn triggers an interferon response in OC stem cells. Patients with OC who exhibit a high expression of endogenous retrovirus have a better chance of surviving their disease, as it increases the efficiency with which cytotoxic immune cells kill EOC, and alters the immune infiltration of tumors (179). It has also been demonstrated that the use of DNMTis to suppress cadherin 13 (CDH13) promoter methylation can lead to an increase in CDH13 expression in OC cells, and a reversal of the malignant phenotype promoted by hsa_circ_0000119 (180). Additionally, several studies have demonstrated that combination therapy with DNMTis and other treatments is more effective than DNMTi monotherapy in the treatment of OC (181-183). In order to overcome platinum resistance in patients with HGSOC, consecutive treatment with the DNMTi, azacytidine, and carboplatin can demethylate and upregulate immune response-related cells (181). Patients with HGSOC have exhibited greater benefits from treatment with DNMTis when used in combination with a histone methyltransferase inhibitor (182). Furthermore, the combined use of a DNMTi and PARP inhibitor has been found to effectively inhibit tumor cell proliferation and migration, while promoting apoptosis, suggesting a potential therapeutic strategy for EOC (183).

Drug-resistant cancer cells have been found to have DNA hypermethylation aberrations, and DNA hypermethylation, produced by chemotherapy, has been proposed as a mechanism and biomarker of drug resistance (174). Patients who have stopped responding to conventional chemotherapy for OC may regain platinum sensitivity following treatment with DNMTis. Patients with recurrent platinum-resistant or poorly responding OC to immunotherapy have been shown to have improved prognostic outcomes and a longer survival time when treated with a combination of DNMTis (184,185). Additionally, in patients with recurrent platinum-resistant OC, the addition of decitabine (a DNA hypomethylating drug) has been shown to enhance the clinical results (186). Patients with OC are more responsive to platinum therapy and have a better prognosis when treated with decitabine plus carboplatin (12).

A previous study using a mouse model demonstrated that DNMTis can also improve survival by increasing immunological signaling, increasing viral defense gene expression in tumor and immune cells, and decreasing the frequency of macrophages and myeloid-derived suppressor cells in the tumor microenvironment (184). Chemokine-like factor (CKLF)-like MARVEL transmembrane domain containing 6 (CMTM6) is overexpressed in OC compared to normal cells due to decreased DNA methylation. Of note, a higher expression of CMTM6 has been shown to be associated with higher immune cell infiltration, which, in turn, can afefct prognosis (187). A recent study demonstrated that the production of pro-inflammatory cytokines/chemokines in human OC cell lines was markedly increased following *in vitro* DNMTi therapy in combination with the editing of transposable factors (188). These results suggest that the therapeutic effects of DNMTis may occur through the modification of the immune response and the OC microenvironment.

Although DNMTis are effective in preventing, treating and determining the prognosis of OC, drug resistance, adverse effects and a poor treatment response continue to be obstacles to the widespread implementation of DNMTi therapeutic regimens. CpG hypermethylation may enhance cancer cell proliferation and alter the response to DNMTis, as previously observed by Giri *et al* (189), which raises doubts about the usefulness of DNMTis in the treatment of patients with OC. However, DNMTis have demonstrated efficacy in reducing or eliminating resistance to chemotherapy and molecular targeting in OC patients. Therefore, further research is required in order to explore the application of DNMTis and verify their viability through clinical studies.

7. Conclusion and future perspectives

Despite the notable advances made in the treatment of OC in recent years, the majority of patients with advanced-stage OC continue to experience recurrence and eventually succumb to chemoresistance. Tumorigenesis, progression and resistance to treatment are predominantly mediated by epigenetic regulation, particularly DNA methylation. The present review aimed to provide an overview of methylation-specific modifications of genes related to OC and their clinical applications, thereby emphasizing the significance of DNA methylation in OC. In general, tumor suppressor genes, such as BRCA1/2, p53, RASSF1A, CHD5, FBP1, ALDH1A2, FOXD3, IGFBP-3, ZNF671, SPARC and MGMT are often found to be underexpressed and hypermethylated in OC tissues (Table II). Conversely, oncogenes, such as HOXA9, CBX8, SLC6A12, AGR2 and GABRP exhibit a high expression and DNA hypomethylation (Table III). In addition to this, the study by Bauerschlag *et al* (190) discovered that the hypomethylation of genes such as growth regulating estrogen receptor binding 1, TGFB induced factor homeobox 1 and transducer of ERBB2, and the hypermethylation of genes such as transmembrane and coiled-coil domains 5, protein tyrosine phosphatase receptor type N and guanylate cyclase 2C, were associated with longer survival periods of patients with OC, suggesting potential prognostic value. The altered DNA methylation of some genes of the classical pathway can also have an impact on the development of OC (Tables IV and V). miRNAs play a more intricate role in the development of OC. Their expression may be downregulated due to gene hypermethylation, such as the expression of miR-152 and miR-148a (191), or they may be overexpressed due to gene hypomethylation, such as miR-21, miR-203 and miR-205 (166) (Table VI). Overall, miRNAs serve as target genes, and investigating whether they are regulated by DNA methylation contributes to the development, diagnosis, staging and treatment resistance of OC, and thus warrants further exploration of their potential clinical applications. Building on comprehensive clinical studies exploring the link between DNA methylation and OC, DNMTis have emerged as a promising therapeutic avenue in clinical settings. They play a pivotal role in overcoming chemoresistance and recurrence in OC. Current therapeutic strategies include the combined use of DNMTis with histone deacetylase inhibitors, DNMTis with PARP inhibitors, among others. These combinations could potentially open up new clinical trial

opportunities for patients with advanced malignant ovarian tumors who are unresponsive to immunotherapy.

Epigenetics, and in particular DNA methylation, is now providing novel and very promising techniques for the discovery of specific biomarkers and their subsequent screening. As previously described by Belsky *et al* (192), the DNA methylation of related genes can be used as a biomarker to predict the rate of aging. Previous studies on DNA methylation in OC have provided critical evidence for understanding ovarian tumorigenesis (61-64). These findings provide potential diagnostic biomarkers and therapeutic targets. However, numerous inconsistencies remain regarding the results of aberrant DNA methylation within these tumor suppressor genes in OC. When analyzing the possible reasons for these inconsistencies, the most significant reason is the sample size. Human samples vary greatly in terms of genetics, environment, lifestyle and individual differences. In a number of studies, the sample size is usually too small due to the huge variation in patients with OC. Future studies are urgently required to address these controversies by analyzing large sample sizes. In addition to this, factors such as the lack of functional studies, differences in the methods of DNA methylation detection used, and different promoter regions for DNA methylation detection may also contribute to the discrepancies. Based on the evidence provided in the present review, targeted DNA methylation inhibitors have promising applications in the treatment of OC. Therefore, it is evident that additional studies are warranted to bridge existing knowledge gaps and reconcile inconsistencies. In light of the known limitations, future research directions, including conducting larger multicenter studies, the development of animal models to determine causality, and the initiation of clinical trials involving methylating or demethylating drugs are proposed. In conclusion, the present review aimed to broaden the understanding of the role of DNA methylation in OC and determine its potential as a biomarker. This could also the focus for future research and further in-depth analysis of this disease. The ultimate goal is to facilitate early diagnosis and treatment, and to promptly address the pressing clinical issues of OC recurrence and chemoresistance.

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Authors' contributions

HD, QG and MF were involved in the writing and preparation of the original draft of the manuscript. QG, HD, FD, MF, YC, JZ, TX, JC, JL and LF were involved in the writing, reviewing and editing of the manuscript. QG, FD, YC, JZ, HD and TX supervised the study. QG, JC, JL, MF and HD were involved in project administration. All authors have read and agreed to the published version of the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Lumish MA, Kohn EC and Tew WP: Top advances of the year: Ovarian cancer. Cancer 130: 837-845, 2024.
2. Tang H, Kulkarni S, Peters C, Eddison J, Al-Ani M and Madhusudan S: The current status of DNA-repair-directed precision oncology strategies in epithelial ovarian cancers. Int J Mol Sci 24: 7293, 2023.
3. Feng J, Xu L, Chen Y, Lin R, Li H and He H: Trends in incidence and mortality for ovarian cancer in China from 1990 to 2019 and its forecasted levels in 30 years. J Ovarian Res 16: 139, 2023.
4. Bodelon C, Killian JK, Sampson JN, Anderson WF, Matsuno R, Brinton LA, Lissowska J, Anglesio MS, Bowtell DDL, Doherty JA, *et al*: Molecular classification of epithelial ovarian cancer based on methylation profiling: Evidence for survival heterogeneity. Clin Cancer Res 25: 5937-5946, 2019.
5. Zhang M, Cheng S, Jin Y, Zhao Y and Wang Y: Roles of CA125 in diagnosis, prediction, and oncogenesis of ovarian cancer. Biochim Biophys Acta Rev Cancer 1875: 188503, 2021.
6. Lheureux S, Braunstein M and Oza AM: Epithelial ovarian cancer: Evolution of management in the era of precision medicine. CA Cancer J Clin 69: 280-304, 2019.
7. Natanzon Y, Goode EL and Cunningham JM: Epigenetics in ovarian cancer. Semin Cancer Biol 51: 160-169, 2018.
8. Moufarrij S, Dandapani M, Arthofer E, Gomez S, Srivastava A, Lopez-Acevedo M, Villagra A and Chiappinelli KB: Epigenetic therapy for ovarian cancer: Promise and progress. Clin Epigenetics 11: 7, 2019.
9. Peng S, Zhang X and Wu Y: Potential applications of DNA methylation testing technology in female tumors and screening methods. Biochim Biophys Acta Rev Cancer 1878: 188941, 2023.
10. Antonino M, Nicolò M, Jerome Renee L, Federico M, Chiara V, Stefano S, Maria S, Salvatore C, Antonio B, Calvo-Henriquez C, *et al*: Single-nucleotide polymorphism in chronic rhinosinusitis: A systematic review. Clin Otolaryngol 47: 14-23, 2022.
11. Matei D and Nephew KP: Epigenetic attire in ovarian cancer: The emperor's new clothes. Cancer Res 80: 3775-3785, 2020.
12. Matei D, Fang F, Shen C, Schilder J, Arnold A, Zeng Y, Berry WA, Huang T and Nephew KP: Epigenetic resensitization to platinum in ovarian cancer. Cancer Res 72: 2197-2205, 2012.
13. Meng H, Cao Y, Qin J, Song X, Zhang Q, Shi Y and Cao L: DNA methylation, its mediators and genome integrity. Int J Biol Sci 11: 604-617, 2015.
14. Singh A, Gupta S and Sachan M: Epigenetic biomarkers in the management of ovarian cancer: Current prospectives. Front Cell Dev Biol 7: 182, 2019.

15. Ma L, Li C, Yin H, Huang J, Yu S, Zhao J, Tang Y, Yu M, Lin J, Ding L and Cui Q: The mechanism of DNA methylation and miRNA in breast cancer. Int J Mol Sci 24: 9360, 2023.
16. Coughlan AY and Testa G: Exploiting epigenetic dependencies in ovarian cancer therapy. Int J Cancer 149: 1732-1743, 2021.
17. Xie W, Sun H, Li X, Lin F, Wang Z and Wang X: Ovarian cancer: Epigenetics, drug resistance, and progression. Cancer Cell Int 21: 434, 2021.
18. Klymenko Y and Nephew KP: Epigenetic crosstalk between the tumor microenvironment and ovarian cancer cells: A therapeutic road less traveled. Cancers (Basel) 10: 295, 2018.
19. Yang Y, Wu L, Shu X, Lu Y, Shu XO, Cai Q, Beeghly-Fadiel A, Li B, Ye F, Berchuck A, *et al*: Genetic data from nearly 63,000 women of european descent predicts DNA methylation biomarkers and epithelial ovarian cancer risk. Cancer Res 79: 505-517, 2019.
20. Lo Riso P, Villa CE, Gasparoni G, Vingiani A, Luongo R, Manfredi A, Jungmann A, Bertolotti A, Borgo F, Garbi A, *et al*: A cell-of-origin epigenetic tracer reveals clinically distinct subtypes of high-grade serous ovarian cancer. Genome Med 12: 94, 2020.
21. Tomar T, Alkema NG, Schreuder L, Meersma GJ, de Meyer T, van Criekinge W, Klip HG, Fiegl H, van Nieuwenhuysen E, Vergote I, *et al*: Methylome analysis of extreme chemoresponsive patients identifies novel markers of platinum sensitivity in high-grade serous ovarian cancer. BMC Med 15: 116, 2017.
22. Feng LY, Yan BB, Huang YZ and Li L: Abnormal methylation characteristics predict chemoresistance and poor prognosis in advanced high-grade serous ovarian cancer. Clin Epigenetics 13: 141, 2021.
23. Yang D, Khan S, Sun Y, Hess K, Shmulevich I, Sood AK and Zhang W: Association of BRCA1 and BRCA2 mutations with survival, chemotherapy sensitivity, and gene mutator phenotype in patients with ovarian cancer. JAMA 306: 1557-1565, 2011.
24. Chan KY, Ozçelik H, Cheung AN, Ngan HY and Khoo US: Epigenetic factors controlling the BRCA1 and BRCA2 genes in sporadic ovarian cancer. Cancer Res 62: 4151-4156, 2002.
25. Stewart C, Ralyea C and Lockwood S: Ovarian cancer: An integrated review. Semin Oncol Nurs 35: 151-156, 2019.
26. Moschetta M, George A, Kaye SB and Banerjee S: BRCA somatic mutations and epigenetic BRCA modifications in serous ovarian cancer. Ann Oncol 27: 1449-1455, 2016.
27. Glajzer J, Castillo-Tong DC, Richter R, Vergote I, Kulbe H, Vanderstichele A, Ruscito I, Trillsch F, Mustea A, Kreuzinger C, *et al*: Impact of BRCA mutation status on tumor dissemination pattern, surgical outcome and patient survival in primary and recurrent high-grade serous ovarian cancer: A multicenter retrospective study by the ovarian cancer therapy-innovative models prolong survival (OCTIPS) consortium. Ann Surg Oncol 30: 35-45, 2023.
28. Jung Y, Hur S, Liu J, Lee S, Kang BS, Kim M and Choi YJ: Peripheral blood BRCA1 methylation profiling to predict familial ovarian cancer. J Gynecol Oncol 32: e23, 2021.
29. Barrett JE, Jones A, Evans I, Reisel D, Herzog C, Chindera K, Kristiansen M, Leavy OC, Manchanda R, Bjørge L, *et al*: The DNA methylome of cervical cells can predict the presence of ovarian cancer. Nat Commun 13: 448, 2022.
30. Wu TI, Huang RL, Su PH, Mao SP, Wu CH and Lai HC: Ovarian cancer detection by DNA methylation in cervical scrapings. Clin Epigenetics 11: 166, 2019.
31. Bartlett TE, Chindera K, McDermott J, Breeze CE, Cooke WR, Jones A, Reisel D, Karegodar ST, Arora R, Beck S, *et al*: Epigenetic reprogramming of fallopian tube fimbriae in BRCA mutation carriers defines early ovarian cancer evolution. Nat Commun 7: 11620, 2016.
32. Ibragimova I and Cairns P: Assays for hypermethylation of the BRCA1 gene promoter in tumor cells to predict sensitivity to PARP-inhibitor therapy. Methods Mol Biol 780: 277-291, 2011.
33. Sahnane N, Carnevali I, Formenti G, Casarin J, Facchi S, Bombelli R, Di Lauro E, Memoli D, Salvati A, Rizzo F, *et al*: BRCA methylation testing identifies a subset of ovarian carcinomas without germline variants that can benefit from PARP inhibitor. Int J Mol Sci 21: 9708, 2020.
34. Taniguchi T, Tischkowitz M, Ameziane N, Hodgson SV, Mathew CG, Joenje H, Mok SC and D'Andrea AD: Disruption of the Fanconi anemia-BRCA pathway in cisplatin-sensitive ovarian tumors. Nat Med 9: 568-574, 2003.
35. Cancer Genome Atlas Research Network: Integrated genomic analyses of ovarian carcinoma. Nature 474: 609-615, 2011.
36. Alsop K, Fereday S, Meldrum C, deFazio A, Emmanuel C, George J, Dobrovic A, Birrer MJ, Webb PM, Stewart C, *et al*: BRCA mutation frequency and patterns of treatment response in BRCA mutation-positive women with ovarian cancer: A report from the Australian ovarian cancer study group. J Clin Oncol 30: 2654-2663, 2012.
37. Mikeska T, Alsop K; Australian Ovarian Cancer Study Group; Mitchell G, Bowtell DD and Dobrovic A: No evidence for PALB2 methylation in high-grade serous ovarian cancer. J Ovarian Res 6: 26, 2013.
38. McAlpine JN, Porter H, Köbel M, Nelson BH, Prentice LM, Kalloger SE, Senz J, Milne K, Ding J, Shah SP, *et al*: BRCA1 and BRCA2 mutations correlate with TP53 abnormalities and presence of immune cell infiltrates in ovarian high-grade serous carcinoma. Mod Pathol 25: 740-750, 2012.
39. Shariati-Kohbanani M, Zare-Bidaki M, Taghavi MM, Taghipour Z, Shabanizadeh A, Kennedy D, Dahim H, Salahshoor MR, Jalili C and Kazemi Arababadi M: DNA methylation and microRNA patterns are in association with the expression of BRCA1 in ovarian cancer. Cell Mol Biol (Noisy-le-grand) 62: 16-23, 2016.
40. Soslow RA, Han G, Park KJ, Garg K, Olvera N, Spriggs DR, Kauff ND and Levine DA: Morphologic patterns associated with BRCA1 and BRCA2 genotype in ovarian carcinoma. Mod Pathol 25: 625-536, 2012.
41. Kraya AA, Maxwell KN, Eiva MA, Wubbenhorst B, Pluta J, Feldman M, Nayak A, Powell DJ Jr, Domchek SM, Vonderheide RH and Nathanson KL: PTEN loss and BRCA1 promoter hypermethylation negatively predict for immunogenicity in BRCA-deficient ovarian cancer. JCO Precis Oncol 6: e2100159, 2022.
42. Ebata T, Yamashita S, Takeshima H, Yoshida H, Kawata Y, Kino N, Yasugi T, Terao Y, Yonemori K, Kato T and Ushijima T: DNA methylation of the immediate upstream region of BRCA1 major transcription start sites is an independent favorable prognostic factor in patients with high-grade serous ovarian cancer. Gynecol Oncol 167: 513-518, 2022.
43. Bai X, Fu Y, Xue H, Guo K, Song Z, Yu Z, Jia T, Yan Y, Zhao L, Mi X, *et al*: BRCA1 promoter hypermethylation in sporadic epithelial ovarian carcinoma: Association with low expression of BRCA1, improved survival and co-expression of DNA methyltransferases. Oncol Lett 7: 1088-1096, 2014.
44. Pradjatmo H: Methylation status and expression of BRCA2 in epithelial ovarian cancers in Indonesia. Asian Pac J Cancer Prev 16: 8599-8604, 2015.
45. Lane DP: p53 and human cancers. Br Med Bull 50: 582-599, 1994.
46. Cancer Genome Atlas Network: Comprehensive molecular portraits of human breast tumours. Nature 490: 61-70, 2012.
47. Chen YC, Young MJ, Chang HP, Liu CY, Lee CC, Tseng YL, Wang YC, Chang WC and Hung JJ: Estradiol-mediated inhibition of DNMT1 decreases p53 expression to induce M2-macrophage polarization in lung cancer progression. Oncogenesis 11: 25, 2022.
48. Suh SO, Chen Y, Zaman MS, Hirata H, Yamamura S, Shahryari V, Liu J, Tabatabai ZL, Kakar S, Deng G, *et al*: MicroRNA-145 is regulated by DNA methylation and p53 gene mutation in prostate cancer. Carcinogenesis 32: 772-778, 2011.
49. Cunningham JM, Winham SJ, Wang C, Weiglt B, Fu Z, Armasu SM, McCauley BM, Brand AH, Chiew YE, Elishaev E, *et al*: DNA methylation profiles of ovarian clear cell carcinoma. Cancer Epidemiol Biomarkers Prev 31: 132-141, 2022.
50. Chmelarova M, Krepinska E, Spacek J, Laco J, Beranek M and Palicka V: Methylation in the p53 promoter in epithelial ovarian cancer. Clin Transl Oncol 15: 160-163, 2013.
51. Kelley KD, Miller KR, Todd A, Kelley AR, Tuttle R and Berberich SJ: YPEL3, a p53-regulated gene that induces cellular senescence. Cancer Res 70: 3566-3575, 2010.
52. Abdollahi A: LOT1 (ZAC1/PLAGL1) and its family members: Mechanisms and functions. J Cell Physiol 210: 16-25, 2007.
53. Su HC, Wu SC, Yen LC, Chiao LK, Wang JK, Chiu YL, Ho CL and Huang SM: Gene expression profiling identifies the role of Zac1 in cervical cancer metastasis. Sci Rep 10: 11837, 2020.
54. Cheng JC, Auersperg N and Leung PC: Inhibition of p53 represses E-cadherin expression by increasing DNA methyltransferase-1 and promoter methylation in serous borderline ovarian tumor cells. Oncogene 30: 3930-3942, 2011.
55. Bin Y, Ding Y, Xiao W and Liao A: RASSF1A: A promising target for the diagnosis and treatment of cancer. Clin Chim Acta 504: 98-108, 2020.

56. Wei B, Wu F, Xing W, Sun H, Yan C, Zhao C, Wang D, Chen X, Chen Y, Li M and Ma J: A panel of DNA methylation biomarkers for detection and improving diagnostic efficiency of lung cancer. Sci Rep 11: 16782, 2021.
57. Tang Q, Cheng J, Cao X, Surowy H and Burwinkel B: Blood-based DNA methylation as biomarker for breast cancer: A systematic review. Clin Epigenetics 8: 115, 2016.
58. Shi H, Li Y, Wang X, Lu C, Yang L, Gu C, Xiong J, Huang Y, Wang S and Lu M: Association between RASSF1A promoter methylation and ovarian cancer: A meta-analysis. PLoS One 8: e76787, 2013.
59. Dammann R, Schagdarsurengin U, Strunnikova M, Rastetter M, Seidel C, Liu L, Tommasi S and Pfeifer GP: Epigenetic inactivation of the Ras-association domain family 1 (RASSF1A) gene and its function in human carcinogenesis. Histol Histopathol 18: 665-677, 2003.
60. Terp SK, Stoico MP, Dybkaer K and Pedersen IS: Early diagnosis of ovarian cancer based on methylation profiles in peripheral blood cell-free DNA: A systematic review. Clin Epigenetics 15: 24, 2023.
61. Rezk NA, Mohamed RH, Alnemr AA and Harira M: Promoter methylation of RASSF1A gene in egyptian patients with ovarian cancer. Appl Biochem Biotechnol 185: 153-162, 2018.
62. Bhagat R, Chadaga S, Premalata CS, Ramesh G, Ramesh C, Pallavi VR and Krishnamoorthy L: Aberrant promoter methylation of the RASSF1A and APC genes in epithelial ovarian carcinoma development. Cell Oncol (Dordr) 35: 473-479, 2012.
63. Xing BL, Li T, Tang ZH, Jiao L, Ge SM, Qiang X and OuYang J: Cumulative methylation alternations of gene promoters and protein markers for diagnosis of epithelial ovarian cancer. Genet Mol Res 14: 4532-4540, 2015.
64. Vo LT, Thuan TB, Thu DM, Uyen NQ, Ha NT and To TV: Methylation profile of BRCA1, RASSF1A and ER in Vietnamese women with ovarian cancer. Asian Pac J Cancer Prev 14: 7713-7718, 2013.
65. Wang H, Cui M, Zhang S, He J, Song L and Chen Y: Relationship between RAS association domain family protein 1A promoter methylation and the clinicopathological characteristics in patients with ovarian cancer: A systematic meta-analysis. Gynecol Obstet Invest 83: 349-357, 2018.
66. Ho CM, Yen TL, Chien TY and Huang SH: Distinct promotor methylation at tumor suppressive genes in ovarian cancer stromal progenitor cells and ovarian cancer and its clinical implication. Am J Cancer Res 12: 5325-5341, 2022.
67. Ho CM, Shih DTB, Hsiao CC, Huang SH, Chang SF and Cheng WF: Gene methylation of human ovarian carcinoma stromal progenitor cells promotes tumorigenesis. J Transl Med 13: 367, 2015.
68. Reyes HD, Devor EJ, Warrier A, Newtson AM, Mattson J, Wagner V, Duncan GN, Leslie KK and Gonzalez-Bosquet J: Differential DNA methylation in high-grade serous ovarian cancer (HGSOC) is associated with tumor behavior. Sci Rep 9: 17996, 2019.
69. Feng Q, Deftereos G, Hawes SE, Stern JE, Willner JB, Swisher EM, Xi L, Drescher C, Urban N and Kiviat N: DNA hypermethylation, Her-2/neu overexpression and p53 mutations in ovarian carcinoma. Gynecol Oncol 111: 320-329, 2008.
70. Xie G, Hu C and Huang M: Methylation status of RASSF1A and clinical efficacy of neoadjuvant therapy in patients with advanced epithelial ovarian cancer. Zhong Nan Da Xue Xue Bao Yi Xue Ban 36: 631-633, 2011 (In Chinese).
71. Giannopoulou L, Chebouti I, Pavlakis K, Kasimir-Bauer S and Lianidou ES: RASSF1A promoter methylation in high-grade serous ovarian cancer: A direct comparison study in primary tumors, adjacent morphologically tumor cell-free tissues and paired circulating tumor DNA. Oncotarget 8: 21429-21443, 2017.
72. S SK, Swamy SN, Premalatha CS, Pallavi VR and Gawari R: Aberrant promoter hypermethylation of RASSF1a and BRCA1 in circulating cell-free tumor DNA serves as a biomarker of ovarian carcinoma. Asian Pac J Cancer Prev 20: 3001-3005, 2019.
73. Marfella CG and Imbalzano AN: The Chd family of chromatin remodelers. Mutat Res 618: 30-40, 2007.
74. Zhao R, Yan Q, Lv J, Huang H, Zheng W, Zhang B and Ma W: CHD5, a tumor suppressor that is epigenetically silenced in lung cancer. Lung Cancer 76: 324-231, 2012.
75. Xie CR, Li Z, Sun HG, Wang FQ, Sun Y, Zhao WX, Zhang S, Zhao WX, Wang XM and Yin ZY: Mutual regulation between CHD5 and EZH2 in hepatocellular carcinoma. Oncotarget 6: 40940-40952, 2015.
76. Qu Y, Dang S and Hou P: Gene methylation in gastric cancer. Clin Chim Acta 424: 53-65, 2013.
77. Du Z, Li L, Huang X, Jin J, Huang S, Zhang Q and Tao Q: The epigenetic modifier CHD5 functions as a novel tumor suppressor for renal cell carcinoma and is predominantly inactivated by promoter CpG methylation. Oncotarget 7: 21618-21630, 2016.
78. Ma Z, Song J, Liu S, Han L, Chen Y, Wang Y, Yu C and Hou L: Decreased expression of the CHD5 gene and its clinicopathological significance in breast cancer: Correlation with aberrant DNA methylation. Oncol Lett 12: 4021-4026, 2016.
79. Dong C, Yuan T, Wu Y, Wang Y, Fan TW, Miriyala S, Lin Y, Yao J, Shi J, Kang T, *et al*: Loss of FBP1 by Snail-mediated repression provides metabolic advantages in basal-like breast cancer. Cancer Cell 23: 316-331, 2013.
80. Li H, Qi Z, Niu Y, Yang Y, Li M, Pang Y, Liu M, Cheng X, Xu M and Wang Z: FBP1 regulates proliferation, metastasis, and chemoresistance by participating in C-MYC/STAT3 signaling axis in ovarian cancer. Oncogene 40: 5938-5949, 2021.
81. Wang Y, Shao F and Chen L: ALDH1A2 suppresses epithelial ovarian cancer cell proliferation and migration by downregulating STAT3. Onco Targets Ther 11: 599-608, 2018.
82. Choi JA, Kwon H, Cho H, Chung JY, Hewitt SM and Kim JH: ALDH1A2 is a candidate tumor suppressor gene in ovarian cancer. Cancers (Basel) 11: 1553, 2019.
83. Zhu L, Zhang S and Jin Y: Foxd3 suppresses NFAT-mediated differentiation to maintain self-renewal of embryonic stem cells. EMBO Rep 15: 1286-1296, 2014.
84. He GY, Hu JL, Zhou L, Zhu XH, Xin SN, Zhang D, Lu GF, Liao WT, Ding YQ and Liang L: The FOXD3/miR-214/MED19 axis suppresses tumour growth and metastasis in human colorectal cancer. Br J Cancer 115: 1367-1378, 2016.
85. Cheng AS, Li MS, Kang W, Cheng VY, Chou JL, Lau SS, Go MY, Lee CC, Ling TK, Ng EK, *et al*: Helicobacter pylori causes epigenetic dysregulation of FOXD3 to promote gastric carcinogenesis. Gastroenterology 144: 122-133.e9, 2013.
86. Luo GF, Chen CY, Wang J, Yue HY, Tian Y, Yang P, Li YK and Li Y: FOXD3 may be a new cellular target biomarker as a hypermethylation gene in human ovarian cancer. Cancer Cell Int 19: 44, 2019.
87. Katsaros D, Yu H, Levesque MA, Danese S, Genta F, Richiardi G, Fracchioli S, Khosravi MJ, Diamandi A, Gordini G, *et al*: IGFBP-3 in epithelial ovarian carcinoma and its association with clinico-pathological features and patient survival. Eur J Cancer 37: 478-485, 2001.
88. Wiley A, Katsaros D, Fracchioli S and Yu H: Methylation of the insulin-like growth factor binding protein-3 gene and prognosis of epithelial ovarian cancer. Int J Gynecol Cancer 16: 210-218, 2006.
89. Torng PL, Lin CW, Chan MW, Yang HW, Huang SC and Lin CT: Promoter methylation of IGFBP-3 and p53 expression in ovarian endometrioid carcinoma. Mol Cancer 8: 120, 2009.
90. Urrutia R: KRAB-containing zinc-finger repressor proteins. Genome Biol 4: 231, 2003.
91. Cheng Y, Geng H, Cheng SH, Liang P, Bai Y, Li J, Srivastava G, Ng MH, Fukagawa T, Wu X, *et al*: KRAB zinc finger protein ZNF382 is a proapoptotic tumor suppressor that represses multiple oncogenes and is commonly silenced in multiple carcinomas. Cancer Res 70: 6516-6526, 2010.
92. Mase S, Shinjo K, Totani H, Katsushima K, Arakawa A, Takahashi S, Lai HC, Lin RI, Chan MWY, Sugiura-Ogasawara M and Kondo Y: ZNF671 DNA methylation as a molecular predictor for the early recurrence of serous ovarian cancer. Cancer Sci 110: 1105-1116, 2019.
93. Yan J, Zhang J, Zhang X, Li X, Li L, Li Z, Chen R, Zhang L, Wu J, Wang X, *et al*: SPARC is down-regulated by DNA methylation and functions as a tumor suppressor in T-cell lymphoma. Exp Cell Res 364: 125-132, 2018.
94. Nagaraju GP and El-Rayes BF: SPARC and DNA methylation: Possible diagnostic and therapeutic implications in gastrointestinal cancers. Cancer Lett 328: 10-17, 2013.
95. Singh A, Gupta S and Sachan M: Evaluation of the diagnostic potential of candidate hypermethylated genes in epithelial ovarian cancer in North Indian population. Front Mol Biosci 8: 719056, 2021.
96. Niskakoski A, Kaur S, Staff S, Renkonen-Sinisalo L, Lassus H, Järvinen HJ, Mecklin JP, Bützow R and Peltomäki P: Epigenetic analysis of sporadic and Lynch-associated ovarian cancers reveals histology-specific patterns of DNA methylation. Epigenetics 9: 1577-1587, 2014.
97. Socha MJ, Said N, Dai Y, Kwong J, Ramalingam P, Trieu V, Desai N, Mok SC and Motamed K: Aberrant promoter methylation of SPARC in ovarian cancer. Neoplasia 11: 126-135, 2009.

98. Gusyatiner O and Hegi ME: Glioma epigenetics: From subclassification to novel treatment options. Semin Cancer Biol 51: 50-58, 2018.
99. Shilpa V, Bhagat R, Premalata CS, Pallavi VR, Ramesh G and Krishnamoorthy L: Relationship between promoter methylation & tissue expression of MGMT gene in ovarian cancer. Indian J Med Res 140: 616-623, 2014.
100. Martínez-Jiménez F, Muiños F, Sentís I, Deu-Pons J, Reyes-Salazar I, Arnedo-Pac C, Mularoni L, Pich O, Bonet J, Kranas H, *et al*: A compendium of mutational cancer driver genes. Nat Rev Cancer 20: 555-572, 2020.
101. Eun Kwon H and Taylor HS: The role of HOX genes in human implantation. Ann N Y Acad Sci 1034: 1-18, 2004.
102. Widschwendter M, Apostolidou S, Jones AA, Fourkala EO, Arora R, Pearce CL, Frasco MA, Ayhan A, Zikan M, Cibula D, *et al*: HOXA methylation in normal endometrium from premenopausal women is associated with the presence of ovarian cancer: A proof of principle study. Int J Cancer 125: 2214-2218, 2009.
103. Wu Q, Lothe RA, Ahlquist T, Silins I, Tropé CG, Micci F, Nesland JM, Suo Z and Lind GE: DNA methylation profiling of ovarian carcinomas and their in vitro models identifies HOXA9, HOXB5, SCGB3A1, and CRABP1 as novel targets. Mol Cancer 6: 45, 2007.
104. Montavon C, Gloss BS, Warton K, Barton CA, Statham AL, Scurry JP, Tabor B, Nguyen TV, Qu W, Samimi G, *et al*: Prognostic and diagnostic significance of DNA methylation patterns in high grade serous ovarian cancer. Gynecol Oncol 124: 582-588, 2012.
105. Singh A, Gupta S, Badarukhiya JA and Sachan M: Detection of aberrant methylation of HOXA9 and HIC1 through multiplex MethyLight assay in serum DNA for the early detection of epithelial ovarian cancer. Int J Cancer 147: 1740-1752, 2020.
106. Faaborg L, Fredslund Andersen R, Waldstrøm M, Høgdall E, Høgdall C, Adimi P, Jakobsen A and Dahl Steffensen K: Analysis of HOXA9 methylated ctDNA in ovarian cancer using sense-antisense measurement. Clin Chim Acta 522: 152-157, 2021.
107. Rusan M, Andersen RF, Jakobsen A and Steffensen KD: Circulating HOXA9-methylated tumour DNA: A novel biomarker of response to poly (ADP-ribose) polymerase inhibition in BRCA-mutated epithelial ovarian cancer. Eur J Cancer 125: 121-129, 2020.
108. van Wijnen AJ, Bagheri L, Badreldin AA, Larson AN, Dudakovic A, Thaler R, Paradise CR and Wu Z: Biological functions of chromobox (CBX) proteins in stem cell self-renewal, lineage-commitment, cancer and development. Bone 143: 115659, 2021.
109. Lin J, Chen L, Wu D, Lin J, Liu B and Guo C: Potential diagnostic and prognostic values of CBX8 Expression in liver hepatocellular carcinoma, kidney renal clear cell carcinoma, and ovarian cancer: A study based on TCGA data mining. Comput Math Methods Med 2022: 1372879, 2022.
110. Li Q, Pan Y, Cao Z and Zhao S: Comprehensive analysis of prognostic value and immune infiltration of chromobox family members in colorectal cancer. Front Oncol 10: 582667, 2020.
111. Sung HY, Yang SD, Park AK, Ju W and Ahn JH: Aberrant hypomethylation of solute carrier family 6 member 12 promoter induces metastasis of ovarian cancer. Yonsei Med J 58: 27-34, 2017.
112. Higa A, Mulot A, Delom F, Bouchecareilh M, Nguyên DT, Boismenu D, Wise MJ and Chevet E: Role of pro-oncogenic protein disulfide isomerase (PDI) family member anterior gradient 2 (AGR2) in the control of endoplasmic reticulum homeostasis. J Biol Chem 286: 44855-44868, 2011.
113. Zhang S, Liu Q, Wei Y, Xiong Y, Gu Y, Huang Y, Tang F and Ouyang Y: Anterior gradient-2 regulates cell communication by coordinating cytokine-chemokine signaling and immune infiltration in breast cancer. Cancer Sci 114: 2238-2253, 2023.
114. He J, Fu Y, Hu J, Chen J and Lou G: Hypomethylation-mediated AGR2 overexpression facilitates cell proliferation, migration, and invasion of lung adenocarcinoma. Cancer Manag Res 13: 5177-5185, 2021.
115. Sung HY, Choi EN, Lyu D, Park AK, Ju W and Ahn JH: Aberrant hypomethylation-mediated AGR2 overexpression induces an aggressive phenotype in ovarian cancer cells. Oncol Rep 32: 815-820, 2014.
116. Chan DW, Lam WY, Chen F, Yung MMH, Chan YS, Chan WS, He F, Liu SS, Chan KKL, Li B and Ngan HYS: Genome-wide DNA methylome analysis identifies methylation signatures associated with survival and drug resistance of ovarian cancers. Clin Epigenetics 13: 142, 2021.
117. Zhan T, Rindtorff N and Boutros M: Wnt signaling in cancer. Oncogene 36: 1461-1473, 2017.
118. Li HJ, Ke FY, Lin CC, Lu MY, Kuo YH, Wang YP, Liang KH, Lin SC, Chang YH, Chen HY, *et al*: ENO1 promotes lung cancer metastasis via HGFR and WNT signaling-driven epithelial-to-mesenchymal transition. Cancer Res 81: 4094-4109, 2021.
119. Xu X, Zhang M, Xu F and Jiang S: Wnt signaling in breast cancer: biological mechanisms, challenges and opportunities. Mol Cancer 19: 165, 2020.
120. Hu W, Li M, Chen Y and Gu X: UBE2S promotes the progression and Olaparib resistance of ovarian cancer through Wnt/β-catenin signaling pathway. J Ovarian Res 14: 121, 2021.
121. Deshmukh A, Arfuso F, Newsholme P and Dharmarajan A: Epigenetic demethylation of sFRPs, with emphasis on sFRP4 activation, leading to Wnt signalling suppression and histone modifications in breast, prostate, and ovary cancer stem cells. Int J Biochem Cell Biol 109: 23-32, 2019.
122. Takada T, Yagi Y, Maekita T, Imura M, Nakagawa S, Tsao SW, Miyamoto K, Yoshino O, Yasugi T, Taketani Y and Ushijima T: Methylation-associated silencing of the Wnt antagonist SFRP1 gene in human ovarian cancers. Cancer Sci 95: 741-744, 2004.
123. Yen HY, Tsao CW, Lin YW, Kuo CC, Tsao CH and Liu CY: Regulation of carcinogenesis and modulation through Wnt/β-catenin signaling by curcumin in an ovarian cancer cell line. Sci Rep 9: 17267, 2019.
124. Ho CM, Lai HC, Huang SH, Chien TY, Lin MC and Chang SF: Promoter methylation of sFRP5 in patients with ovarian clear cell adenocarcinoma. Eur J Clin Invest 40: 310-318, 2010.
125. Ho CM, Huang CJ, Huang CY, Wu YY, Chang SF and Cheng WF: Promoter methylation status of HIN-1 associated with outcomes of ovarian clear cell adenocarcinoma. Mol Cancer 11: 53, 2012.
126. Lin HW, Fu CF, Chang MC, Lu TP, Lin HP, Chiang YC, Chen CA and Cheng WF: CDH1, DLEC1 and SFRP5 methylation panel as a prognostic marker for advanced epithelial ovarian cancer. Epigenomics 10: 1397-1413, 2018.
127. Su HY, Lai HC, Lin YW, Liu CY, Chen CK, Chou YC, Lin SP, Lin WC, Lee HY and Yu MH: Epigenetic silencing of SFRP5 is related to malignant phenotype and chemoresistance of ovarian cancer through Wnt signaling pathway. Int J Cancer 127: 555-567, 2010.
128. Kumar D, Patel SA, Hassan MK, Mohapatra N, Pattanaik N and Dixit M: Reduced IQGAP2 expression promotes EMT and inhibits apoptosis by modulating the MEK-ERK and p38 signaling in breast cancer irrespective of ER status. Cell Death Dis 12: 389, 2021.
129. Deng Z, Wang L, Hou H, Zhou J and Li X: Epigenetic regulation of IQGAP2 promotes ovarian cancer progression via activating Wnt/β-catenin signaling. Int J Oncol 48: 153-160, 2016.
130. Ge YX, Wang CH, Hu FY, Pan LX, Min J, Niu KY, Zhang L, Li J and Xu T: New advances of TMEM88 in cancer initiation and progression, with special emphasis on Wnt signaling pathway. J Cell Physiol 233: 79-87, 2018.
131. de Leon M, Cardenas H, Vieth E, Emerson R, Segar M, Liu Y, Nephew K and Matei D: Transmembrane protein 88 (TMEM88) promoter hypomethylation is associated with platinum resistance in ovarian cancer. Gynecol Oncol 142: 539-547, 2016.
132. Yang L, Pang Y and Moses HL: TGF-beta and immune cells: An important regulatory axis in the tumor microenvironment and progression. Trends Immunol 31: 220-227, 2010.
133. Morikawa M, Derynck R and Miyazono K: TGF-β and the TGF-β family: Context-dependent roles in cell and tissue physiology. Cold Spring Harb Perspect Biol 8: a021873, 2016.
134. Matsumura N, Huang Z, Mori S, Baba T, Fujii S, Konishi I, Iversen ES, Berchuck A and Murphy SK: Epigenetic suppression of the TGF-beta pathway revealed by transcriptome profiling in ovarian cancer. Genome Res 21: 74-82, 2011.
135. Cardenas H, Vieth E, Lee J, Segar M, Liu Y, Nephew KP and Matei D: TGF-β induces global changes in DNA methylation during the epithelial-to-mesenchymal transition in ovarian cancer cells. Epigenetics 9: 1461-1472, 2014.
136. Chou JL, Su HY, Chen LY, Liao YP, Hartman-Frey C, Lai YH, Yang HW, Deatherage DE, Kuo CT, Huang YW, *et al*: Promoter hypermethylation of FBXO32, a novel TGF-beta/SMAD4 target gene and tumor suppressor, is associated with poor prognosis in human ovarian cancer. Lab Invest 90: 414-425, 2010.

137. Chou JL, Huang RL, Shay J, Chen LY, Lin SJ, Yan PS, Chao WT, Lai YH, Lai YL, Chao TK, *et al*: Hypermethylation of the TGF-β target, ABCA1 is associated with poor prognosis in ovarian cancer patients. Clin Epigenetics 7: 1, 2015.
138. Wegner M: All purpose Sox: The many roles of Sox proteins in gene expression. Int J Biochem Cell Biol 42: 381-390, 2010.
139. Shonibare Z, Monavarian M, O'Connell K, Altomare D, Shelton A, Mehta S, Jaskula-Sztul R, Phaeton R, Starr MD, Whitaker R, *et al*: Reciprocal SOX2 regulation by SMAD1-SMAD3 is critical for anoikis resistance and metastasis in cancer. Cell Rep 40: 111066, 2022.
140. Ween MP, Oehler MK and Ricciardelli C: Transforming growth factor-beta-induced protein (TGFBI)/(βig-H3): A matrix protein with dual functions in ovarian cancer. Int J Mol Sci 13: 10461-10477, 2012.
141. Wang N, Zhang H, Yao Q, Wang Y, Dai S and Yang X: TGFBI promoter hypermethylation correlating with paclitaxel chemoresistance in ovarian cancer. J Exp Clin Cancer Res 31: 6, 2012.
142. Ho CM, Lin MC, Huang SH, Huang CJ, Lai HC, Chien TY and Chang SF: PTEN promoter methylation and LOH of 10q22-23 locus in PTEN expression of ovarian clear cell adenocarcinomas. Gynecol Oncol 112: 307-313, 2009.
143. Ediriweera MK, Tennekoon KH and Samarakoon SR: Role of the PI3K/AKT/mTOR signaling pathway in ovarian cancer: Biological and therapeutic significance. Semin Cancer Biol 59: 147-160, 2019.
144. Li M, Balch C, Montgomery JS, Jeong M, Chung JH, Yan P, Huang TH, Kim S and Nephew KP: Integrated analysis of DNA methylation and gene expression reveals specific signaling pathways associated with platinum resistance in ovarian cancer. BMC Med Genomics 2: 34, 2009.
145. Li GN, Zhao XJ, Wang Z, Luo MS, Shi SN, Yan DM, Li HY, Liu JH, Yang Y, Tan JH, *et al*: Elaiophylin triggers paraptosis and preferentially kills ovarian cancer drug-resistant cells by inducing MAPK hyperactivation. Signal Transduct Target Ther 7: 317, 2022.
146. Walch A, Specht K, Braselmann H, Stein H, Siewert JR, Hopt U, Höfler H and Werner M: Coamplification and coexpression of GRB7 and ERBB2 is found in high grade intraepithelial neoplasia and in invasive Barrett's carcinoma. Int J Cancer 112: 747-753, 2004.
147. Chen K, Liu MX, Mak CS, Yung MM, Leung TH, Xu D, Ngu SF, Chan KK, Yang H, Ngan HY and Chan DW: Methylation-associated silencing of miR-193a-3p promotes ovarian cancer aggressiveness by targeting GRB7 and MAPK/ERK pathways. Theranostics 8: 423-436, 2018.
148. Sung HY, Yang SD, Ju W and Ahn JH: Aberrant epigenetic regulation of GABRP associates with aggressive phenotype of ovarian cancer. Exp Mol Med 49: e335, 2017.
149. He L and Hannon GJ: MicroRNAs: Small RNAs with a big role in gene regulation. Nat Rev Genet 5: 522-531, 2004.
150. Kuhlmann JD, Rasch J, Wimberger P and Kasimir-Bauer S: microRNA and the pathogenesis of ovarian cancer-a new horizon for molecular diagnostics and treatment? Clin Chem Lab Med 50: 601-615, 2012.
151. Cairns RA: Drivers of the Warburg phenotype. Cancer J 21: 56-61, 2015.
152. Zhou Y, Zheng X, Lu J, Chen W, Li X and Zhao L: Ginsenoside 20(S)-Rg3 inhibits the Warburg effect via modulating DNMT3A/MiR-532-3p/HK2 pathway in ovarian cancer cells. Cell Physiol Biochem 45: 2548-2559, 2018.
153. Zhang S, Pei M, Li Z, Li H, Liu Y and Li J: Double-negative feedback interaction between DNA methyltransferase 3A and microRNA-145 in the Warburg effect of ovarian cancer cells. Cancer Sci 109: 2734-2745, 2018.
154. Li J, Zhang S, Zou Y, Wu L, Pei M and Jiang Y: miR-145 promotes miR-133b expression through c-myc and DNMT3A-mediated methylation in ovarian cancer cells. J Cell Physiol 235: 4291-4301, 2020.
155. Teng Y, Zuo X, Hou M, Zhang Y, Li C, Luo W and Li X: A double-negative feedback interaction between MicroRNA-29b and DNMT3A/3B contributes to ovarian cancer progression. Cell Physiol Biochem 39: 2341-2352, 2016.
156. Chhabra R, Rockfield S, Guergues J, Nadeau OW, Hill R, Stevens SM Jr and Nanjundan M: Global miRNA/proteomic analyses identify miRNAs at 14q32 and 3p21, which contribute to features of chronic iron-exposed fallopian tube epithelial cells. Sci Rep 11: 6270, 2021.
157. Chen Q, Wang Y, Dang H and Wu X: MicroRNA-148a-3p inhibits the proliferation of cervical cancer cells by regulating the expression levels of DNMT1 and UTF1. Oncol Lett 22: 617, 2021.
158. Wu YH, Huang YF, Wu PY, Chang TH, Huang SC and Chou CY: The downregulation of miR-509-3p expression by collagen type XI alpha 1-regulated hypermethylation facilitates cancer progression and chemoresistance via the DNA methyltransferase 1/Small ubiquitin-like modifier-3 axis in ovarian cancer cells. Res Sq: rs.3.rs-2592453, 2023.
159. Han X, Zhen S, Ye Z, Lu J, Wang L, Li P, Li J, Zheng X, Li H, Chen W, *et al*: A feedback loop between miR-30a/c-5p and DNMT1 mediates cisplatin resistance in ovarian cancer cells. Cell Physiol Biochem 41: 973-986, 2017.
160. Vera O, Jimenez J, Pernia O, Rodriguez-Antolin C, Rodriguez C, Sanchez Cabo F, Soto J, Rosas R, Lopez-Magallon S, Esteban Rodriguez I, *et al*: DNA methylation of miR-7 is a mechanism involved in platinum response through MAFG overexpression in cancer cells. Theranostics 7: 4118-4134, 2017.
161. Li X, Pan Q, Wan X, Mao Y, Lu W, Xie X and Cheng X: Methylation-associated Has-miR-9 deregulation in paclitaxel-resistant epithelial ovarian carcinoma. BMC Cancer 15: 509, 2015.
162. Yang C, Cai J, Wang Q, Tang H, Cao J, Wu L and Wang Z: Epigenetic silencing of miR-130b in ovarian cancer promotes the development of multidrug resistance by targeting colony-stimulating factor 1. Gynecol Oncol 124: 325-334, 2012.
163. Xu S, Fu GB, Tao Z, OuYang J, Kong F, Jiang BH, Wan X and Chen K: MiR-497 decreases cisplatin resistance in ovarian cancer cells by targeting mTOR/P70S6K1. Oncotarget 6: 26457-26471, 2015.
164. Han X, Liu D, Zhou Y, Wang L, Hou H, Chen H, Zhang L, Chen W, Li X and Zhao L: The negative feedback between miR-143 and DNMT3A regulates cisplatin resistance in ovarian cancer. Cell Biol Int 45: 227-237, 2021.
165. He L, Zhu W, Chen Q, Yuan Y, Wang Y, Wang J and Wu X: Ovarian cancer cell-secreted exosomal miR-205 promotes metastasis by inducing angiogenesis. Theranostics 9: 8206-8220, 2019.
166. Iorio MV, Visone R, Di Leva G, Donati V, Petrocca F, Casalini P, Taccioli C, Volinia S, Liu CG, Alder H, *et al*: MicroRNA signatures in human ovarian cancer. Cancer Res 67: 8699-8707, 2007.
167. Loginov VI, Pronina IV, Filippova EA, Burdennyy AM, Lukina SS, Kazubskaya TP, Uroshlev LA, Fridman MV, Brovkina OI, Apanovich NV, *et al*: Aberrant methylation of 20 miRNA genes specifically involved in various steps of ovarian carcinoma spread: From primary tumors to peritoneal macroscopic metastases. Int J Mol Sci 23: 1300, 2022.
168. Deng Y, Zhao F, Hui L, Li X, Zhang D, Lin W, Chen Z and Ning Y: Suppressing miR-199a-3p by promoter methylation contributes to tumor aggressiveness and cisplatin resistance of ovarian cancer through promoting DDR1 expression. J Ovarian Res 10: 50, 2017.
169. Schmid G, Notaro S, Reimer D, Abdel-Azim S, Duggan-Peer M, Holly J, Fiegl H, Rössler J, Wiedemair A, Concin N, *et al*: Expression and promotor hypermethylation of miR-34a in the various histological subtypes of ovarian cancer. BMC Cancer 16: 102, 2016.
170. Zuberi M, Khan I, Mir R, Gandhi G, Ray PC and Saxena A: Utility of serum miR-125b as a diagnostic and prognostic indicator and its alliance with a panel of tumor suppressor genes in epithelial ovarian cancer. PLoS One 11: e0153902, 2016.
171. He J, Xu Q, Jing Y, Agani F, Qian X, Carpenter R, Li Q, Wang XR, Peiper SS, Lu Z, *et al*: Reactive oxygen species regulate ERBB2 and ERBB3 expression via miR-199a/125b and DNA methylation. EMBO Rep 13: 1116-1122, 2012.
172. Ye Z, Li J, Han X, Hou H, Chen H, Zheng X, Lu J, Wang L, Chen W, Li X and Zhao L: TET3 inhibits TGF-β1-induced epithelial-mesenchymal transition by demethylating miR-30d precursor gene in ovarian cancer cells. J Exp Clin Cancer Res 35: 72, 2016.
173. Vogt M, Munding J, Grüner M, Liffers ST, Verdoodt B, Hauk J, Steinstraesser L, Tannapfel A and Hermeking H: Frequent concomitant inactivation of miR-34a and miR-34b/c by CpG methylation in colorectal, pancreatic, mammary, ovarian, urothelial, and renal cell carcinomas and soft tissue sarcomas. Virchows Arch 458: 313-322, 2011.
174. Cai Y, Tsai HC, Yen RC, Zhang YW, Kong X, Wang W, Xia L and Baylin SB: Critical threshold levels of DNA methyltransferase 1 are required to maintain DNA methylation across the genome in human cancer cells. Genome Res 27: 533-544, 2017.

175. Li H, Lei Y, Li S, Li F and Lei J: MicroRNA-20a-5p inhibits the autophagy and cisplatin resistance in ovarian cancer via regulating DNMT3B-mediated DNA methylation of RBP1. Reprod Toxicol 109: 93-100, 2022.
176. Ye Z, Jiang Y and Wu J: DNMT3B attenuated the inhibition of TET3 on epithelial-mesenchymal transition in TGF-β1-induced ovarian cancer by methylating the TET3 promoter. Reprod Biol 22: 100701, 2022.
177. Del Castillo Falconi VM, Díaz-Chávez J, Torres-Arciga K, Luna-Maldonado F, Gudiño-Gomez AA, Pedroza-Torres A, Castro-Hernández C, Cantú de León D and Herrera LA: Expression of DNA methyltransferase 3B isoforms is associated with DNA satellite 2 hypomethylation and clinical prognosis in advanced high-grade serous ovarian carcinoma. Int J Mol Sci 23: 12759, 2022.
178. Lyko F and Brown R: DNA methyltransferase inhibitors and the development of epigenetic cancer therapies. J Natl Cancer Inst 97: 1498-1506, 2005.
179. Natoli M, Gallon J, Lu H, Amgheib A, Pinato DJ, Mauri FA, Marafioti T, Akarca AU, Ullmo I, Ip J, *et al*: Transcriptional analysis of multiple ovarian cancer cohorts reveals prognostic and immunomodulatory consequences of ERV expression. J Immunother Cancer 9: e001519, 2021.
180. Ma G, Li Y, Meng F, Sui C, Wang Y and Cheng D: Hsa_circ_0000119 promoted ovarian cancer development via enhancing the methylation of CDH13 by sponging miR-142-5p. J Biochem Mol Toxicol 37: e23264, 2023.
181. Wong-Brown MW, van der Westhuizen A and Bowden NA: Sequential azacitidine and carboplatin induces immune activation in platinum-resistant high-grade serous ovarian cancer cell lines and primes for checkpoint inhibitor immunotherapy. BMC Cancer 22: 100, 2022.
182. Liu M, Thomas SL, DeWitt AK, Zhou W, Madaj ZB, Ohtani H, Baylin SB, Liang G and Jones PA: Dual inhibition of DNA and histone methyltransferases increases viral mimicry in ovarian cancer cells. Cancer Res 78: 5754-5766, 2018.
183. Shim JI, Ryu JY, Jeong SY, Cho YJ, Choi JJ, Hwang JR, Choi JY, Sa JK and Lee JW: Combination effect of poly (ADP-ribose) polymerase inhibitor and DNA demethylating agents for treatment of epithelial ovarian cancer. Gynecol Oncol 165: 270-280, 2022.
184. McDonald JI, Diab N, Arthofer E, Hadley M, Kanholm T, Rentia U, Gomez S, Yu A, Grundy EE, Cox O, *et al*: Epigenetic therapies in ovarian cancer alter repetitive element expression in a TP53-dependent manner. Cancer Res 81: 5176-5189, 2021.
185. Steele N, Finn P, Brown R and Plumb JA: Combined inhibition of DNA methylation and histone acetylation enhances gene re-expression and drug sensitivity in vivo. Br J Cancer 100: 758-763, 2009.
186. Fang F, Balch C, Schilder J, Breen T, Zhang S, Shen C, Li L, Kulesavage C, Snyder AJ, Nephew KP and Matei DE: A phase 1 and pharmacodynamic study of decitabine in combination with carboplatin in patients with recurrent, platinum-resistant, epithelial ovarian cancer. Cancer 116: 4043-4053, 2010.
187. Yin B, Ding J, Hu H, Yang M, Huang B, Dong W, Li F and Han L: Overexpressed CMTM6 improves prognosis and associated with immune infiltrates of ovarian cancer. Front Mol Biosci 9: 769032, 2022.
188. Gomez S, Cox OL, Walker RR III, Rentia U, Hadley M, Arthofer E, Diab N, Grundy EE, Kanholm T, McDonald JI, *et al*: Inhibiting DNA methylation and RNA editing upregulates immunogenic RNA to transform the tumor microenvironment and prolong survival in ovarian cancer. J Immunother Cancer 10: e004974, 2022.
189. Giri AK and Aittokallio T: DNMT inhibitors increase methylation in the cancer genome. Front Pharmacol 10: 385, 2019.
190. Bauerschlag DO, Ammerpohl O, Bräutigam K, Schem C, Lin Q, Weigel MT, Hilpert F, Arnold N, Maass N, Meinhold-Heerlein I and Wagner W: Progression-free survival in ovarian cancer is reflected in epigenetic DNA methylation profiles. Oncology 80: 12-20, 2011.
191. Khajehnoori S, Zarei F, Mazaheri M and Dehghani-Firoozabadi A: Epidrug modulated expression of MiR-152 and MiR-148a reverse cisplatin resistance in ovarian cancer cells: An experimental in-vitro study. Iran J Pharm Res 19: 509-519, 2020.
192. Belsky DW, Caspi A, Corcoran DL, Sugden K, Poulton R, Arseneault L, Baccarelli A, Chamarti K, Gao X, Hannon E, *et al*: DunedinPACE, a DNA methylation biomarker of the pace of aging. Elife 11: e73420, 2022.